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PHYSIOLOGIC SPECIALIZATION OF THE PARASITIC FUNGI. II[1]

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Investigations of physiologic specialization in plant pathogens, especially those which attack cereals, vegetable crops and other economic plant groups, continue to occupy a foremost place in plant pathology. During the past ten years many workers in various parts of the world have contributed to our knowledge of the extent of host specialization and have emphasized the importance of the recognition of this phenomenon in the practical breeding for disease-resistant varieties of crop plants.

CEREAL RUSTS

Puccinia graminis tritici. In 1934, 127 races of the stem rust of wheat were listed for the entire world. Since then, many additions have been made, a total of 189 being recorded in 1944 (127). New races have been discovered in Poland (34), Germany (50, 53, 74), Italy and Italian East Africa (119, 120), Portugal (21), Australia and New Zealand (166), Argentina, Brazil, Chile and Uruguay (150, 153) and in China (145), as well as additional races recorded by American investigators.

A virulent race has been found on Khapli emmer in Peru (35). It is designated as No. 189 and is very locally distributed, not having been isolated from other parts of South America or elsewhere. Since Khapli emmer has been extremely resistant to other known races of the rust, the new race may be an important one, for, if introduced into the spring-wheat region of the United States, it might be disastrous to the new wheat varieties which depend upon Khapli for resistance.

Interesting changes have taken place in the occurrence of specific races of the stem rust of wheat. Race 56, which was first

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found in 1928, has rapidly increased, in 1938 about 60% of the stem rust isolates belonging to this race (125). In contrast, race 34 has greatly decreased since 1934. Data from the annual physiologic race surveys made in the United States for more than 20 years have been recorded (129). During 1930–1941, five races—Nos. 17, 36, 38, 49 and 56—ranked first in prevalence. During 1930–1934, races 36, 38 and 49 seemed to be the most important, while in 1935–1939, race 56 ranked first. It is especially emphasized that the increase of race 56 during the period was particularly significant, constituting about 90% of the rust in the Mississippi Basin. The predominance of race 56 in Canada may be one of the factors in the reduction of the cultivation of the Ceres variety of wheat (70). Observations on the prevalent races in southern Mexico have been recorded (130).

A special study of race 15B, which has been identified in many collections from different parts of the United States, has been made (45). It appears to be a potentially dangerous one, since many of the wheat varieties and hybrids being bred in the Mississippi Valley are susceptible to it, and should the newer hybrids be introduced, it might become prevalent and destructive.

The origin of abnormal rust characteristics through the inbreeding of physiologic races has been investigated (68). Observations have been made on such peculiarities as greyish-brown, orange or white urediospores; decreased vigor of sporulation; decreased virulence; increased sensitivity to high temperatures; inability to produce aecia on barberry; and development of urediospores and teliospores by some strains that have partially lost the ability to produce aecia. The crossing of races and selfing of the hybrid generations may lead to recombinations of pathogenic characters resulting in the formation of new physiologic races (69).

Teliospores obtained on *Agrostis* were used to inoculate barberry, and the aeciospores from barberry infected barley, the culture proving to be race 11 of *Puccinia graminis tritici.* Crosses between this collection and races of *P. g. tritici, P. g. secalis* and *P. g. agrostidis* yielded additional races of each of these groups, one of the *tritici* races, No. 160, being different from any previously identified (16).

Two varieties of barley were tested with 19 races of *Puccinia graminis tritici* and one of *P. g. secalis,* both varieties being sus-

ceptible to the former and resistant to the latter. Other hybrids and varieties of barley were tested, reacting in the seedling and in the adult stage in the same manner to the races used (63).

The recently described species of wheat, *Triticum timopheevi,* has been reported as very rust-resistant. It has been inoculated with 20 physiologic races, without any infection (94). Its resistance has been transferred to the common winter wheat through hybridization (118). More recently, it is reported that *T. timopheevi* is susceptible to races 15B and 189, the newly discovered virulent race from Peru (44).

Puccinia graminis avenae. A stem rust collected on *Avena fatua* infected 18 species belonging to nine genera, distributed in three tribes of grasses, and the rust culture was identified as a strain or race of *Puccinia graminis avenae.* Rust from *Poa ampla* infected 34 species of grasses in 14 genera and five tribes, this rust also probably belonging to the *avenae* group. However, it did not infect 16 out of 17 varieties of cultivated oats, thus indicating a new physiologic race. A culture from *Agropyron spicatum* infected seven species, representing three genera limited to one tribe of grasses and does not seem to belong to any of the known races, not being related to *Puccinia graminis secalis* or *P. g. tritici* (28).

The reaction of oat varieties in the seedling and maturing stages to five physiologic races—1, 2, 5, 7 and 10—has been studied. Races 2 and 5 had been significant in the stem rust epidemics during 1921–1935 (79). Twelve physiologic races in Canada were recorded during the period 1921–1943, races 1, 2 and 5 being the most prevalent, especially in the three Prairie Provinces where the barberry is practically absent. The effect of environment on the reaction of an oat variety to races 1 and 11 is also described. The type 1 infection in summer is frequently replaced by type X. A temperature of about 80° does not appreciably affect the reaction of White Tartar and Richland to the rust. However, the resistance of other varieties, derived from certain crosses, is broken down (93).

Attention is called to the possible importance of race 8 which, along with race 10, causes heavy infection on Richland, Vicland, Boone, Tama and other varieties of oats which are derived from Victoria × Richland crosses (128). Two races of oat rust are recorded for Argentina, practically all of the local varieties of oats

being susceptible. Introduced varieties, as Rainbow, Richland, Iogold, Green Russian and Hawkeye, showed resistance. Various grasses, including species of *Dactylis, Poa, Bromus, Briza* and *Phalaris,* showed some susceptibility to the stem rust (158).

Puccinia triticina. Many investigators have added to our knowledge of specialization in the leaf rust of wheat. In 1933, 53 known races in various parts of the world were recorded (81). By 1942 the number had been increased to 129 (71). Additional races have been found in England, Wales and Portugal (107), Poland (100), various other parts of Europe (163), the Odessa region of Russia (39), the vicinity of Leningrad (97), the Saratoff region (169) and also widely distributed over the U.S.S.R. (101). Specialization has also been recorded in Libya and Abyssinia (121), Brazil and Chile (152, 157), India (87) and Japan (5).

Puccinia glumarum. Additional evidence of specialization of stripe rust of wheat and barley has been supplied. Several races have been reported from various parts of Europe (132, 136), from India (87) and China (23), mostly from the Yunnan Province. Practically all of the native wheat varieties were susceptible to the rust. However, a few foreign introductions were somewhat resistant, and might provide a basis for breeding resistant varieties of wheat and barley. Observations made on the occurrence and distribution of this rust in eastern Harz indicate that *Agropyron caninum* may be an important host for races of the rust which pass over to wheat and barley (6).

Puccinia dispersa. The study of specialization in rusts and other diseases of rye is complicated by the fact that it is difficult to obtain genotypically pure rye varieties for inoculation. Leaf rust causes pustules on some wheat varieties and necrosis or chlorosis on others, these reactions making it possible to differentiate two races among ten monospore lines of the brown rust of rye, using 28 varieties of wheat.

Puccinia coronata. In 1930, 33 specialized races of the crown rust of oats were recorded in Germany (33), and in 1933 the same number of races from the United States and Canada (92). Since then, additional contributions to the specialization of this rust have been published. Seven distinct varieties of *Puccinia coronata* on grass hosts in England, namely, *alopecuri, arrhenatheri, avenae, calamagrostidis, festucae, lolii* and *holci,* have been recorded. Some

of these are sharply specialized, while others are not. Four races of the *avenae* group are listed, as well as a new one from Portugal (11). Four races of this rust have been described from Argentina. (151).

OTHER RUSTS

Other species of rust have been investigated from the standpoint of physiologic specialization. Three races of *Uromyces graminis* were obtained from over-wintered teliospores on *Melica ciliata.* A sixth race has been added to the five known races of *U. dactylidis,* also three new physiologic races of *U. festucae* and an eighth race of *U. poae* have been recorded (37, 38).

The flax rust, *Melampsora lini,* has been studied extensively. In 1935, 14 races were differentiated in the United States and Canada (31), and ten additional races in 1940, three of these being obtained from material of South America (32). Four distinct races have been differentiated from collections made in Holland, Sweden and Germany (135), and six races have been listed in Australia (167).

In 1935, two races of the bean rust, *Uromyces phaseoli typica,* were differentiated (47), and in 1939 two collections from Florida and Washington were separated as distinct (22). In 1939, 13 physiologic races of this rust were isolated from the United States and Hawaii, two or more occurring in a given locality, varying in their predominance from year to year (46). Later, an additional strain was described from Hawaii (96). In a more complete investigation, 20 physiologic races have been described, based on the use of seven differential host varieties (48).

Specialized races of other rusts have also been found, *e.g.,* in *Gymnosporangium clavipes* on the apple (91); four specialized races of sunflower rust, *Puccinia helianthi,* on *Helianthus annuus, H. petiolaris, H. tuberosus* and *H. subtuberosus* (10); and two races of *Puccinia iridis* in 1934, one being distinguished by the susceptibility of *Iris fulva* and *I. foliosa,* while the other was unable to attack these species of iris (83). Another race of the iris rust was found on the bearded iris at Berkeley, California (84). Two distinct races of snapdragon rust were found in California, several snapdragons resistant to race 1 proving to be very susceptible to race 2 (171). Breeding experiments in Massachusetts for re-

sistance to snapdragon rust, however, revealed no evidence of physiologic races (168).

CEREAL SMUTS

Oat smuts. Several publications recorded evidence of further specialization in the loose and covered smuts of oats. In 1940, 29 specialized races of the loose smut and 14 of the covered were listed (105). Three additional races of each of the smuts were recorded by another investigator (140). An interesting race of loose smut which was able to infect Black Mesdag oats was described in 1938 (160). More recently, an additional race of loose smut was recorded which was interesting because of its ability to attack the Victoria oat variety which hitherto had been resistant to all known races of both loose and covered smuts (106). It has been suggested that loose smut of oats, *Ustilago avenae,* and the smut on *Arrhenatherum elatius, U. perennans,* were specifically identical. Oat varieties were successfully infected with spores from the grass. Hybrids between the two smuts were made, but tall oatgrass remained resistant to both loose smut from oats and the hybrids between the two smuts (29).

Barley smuts. Two physiologic races of the loose smut of barley, *Ustilago nuda,* one attacking a spring barley and the other a winter variety, have been described (141). Several additional races of *U. nigra* have been listed (72, 137, 139). Further specialization in *U. hordei* has been demonstrated, eight races being found in different parts of the United States (138) and evidence of specialization for races in Alberta, Canada (117). In China (174) five races have been recorded. One of the difficult problems in the covered smut of barley is the development of effective methods for the infection of susceptible varieties.

The occurrence of *Ustilago hordei* and *U. nigra* on forage grasses has been described. The covered smut of barley, *U. hordei,* was collected on *Agropyron cristatum* and *Elymus glaucus jepsoni,* and three collections from these hosts infected Beldi Giant and Trebi barley. Canadian oats, however, was not infected by this smut. It was concluded that the covered smut on the grasses has a close relationship to *U. hordei.* Twenty-five species of *Agropyron, Elymus, Hordeum, Lolium* and *Sitanion* were inoculated, and high infections obtained on many of them. Several grass

species were also inoculated with *U. nigra,* and 30% to 50% infection was obtained on *Elymus canadensis, Hordeum nodosum* and *Sitanion jubatum* (24).

Wheat smuts. Additional investigations have been carried out with the two species of *Tilletia* on wheat. In 1936, two races of *Tilletia tritici* were recorded (57), and in 1937, eleven races of *T. tritici* and eight of *T. laevis.* In 1942, five additional races of *T. tritici* and *T. laevis* were noted. Studies of some of these races of the two species were made, recording differences in the size, shape and water-absorption properties of the bunt-ball; the size, echinulation and color of the chlamydospore; the capacity to stunt the host or to stimulate excessive tillering; and the incomplete infection and intensification of the pigmentation of the glumes. One race of *T. tritici* gave rise to a different one, indicating the lack of stability on the part of the races (59).

In Russia, five varieties of *Tilletia tritici* have been distinguished on morphological grounds—dark brown, light brown, typical, whitish-brown and spiculate spores—and also three forms of *T. laevis*—dark brown, light brown and greyish-brown spores, a new variety also being described (122).

In 1935, four races of *T. laevis* and nine of *T. tritici* were recorded in Argentina, three of the latter infecting rye (95). In 1938 several races of *T. tritici* were listed for Australia (15), and five races of *Tilletia tritici* and five of *T. laevis* were recorded in Rumania (114).

A series of resistant varieties of wheat was inoculated with six races of *Tilletia tritici* and three of *T. laevis* (7). No significant increase in the percentage of bunt infection was noted when they were inoculated with their own smut over a three-year period, except in one case which may have been due to a segregation in the smut's capacity for infection. Another observer has recorded a marked decline in the pathogenicity of a physiologic race of *T. laevis* when grown on a susceptible variety over a period of years, results at marked variance from those obtained by others (162). The two species of *Tilletia* on wheat have been hybridized, and distinct races developed among the hybrids (56).

It has also been found that both *Tilletia laevis* and *T. tritici* are able to infect species of grasses in the tribe *Hordeae,* including *Agropyron inerme, A. spicatum, A. trichophorum* and *Sitanion jubatum* (25).

Very little work has been done on the loose smut of wheat. However, four races have been differentiated in Manitoba (40).

Flag smut of wheat. In 1936, five specialized races of flag smut of wheat, *Urocystis tritici,* were recorded in China (176), and in 1945, seven additional ones were listed (177). In the United States two races were differentiated in 1943 (58).

The relations of *Urocystis agropyri, U. occulta* and *U. tritici* have been investigated. Twelve species of grass belonging to the genera *Agropyron, Elymus* and *Hordeum* are more or less susceptible to the flag smut of wheat, and four species of grass are somewhat susceptible to the flag smut of rye—*Agropyron caninum, A. inerme, Elymus canadensis* and *E. triticoides.* Among the collections of grass smut studied, three physiologic races were differentiated. It was further noted that wheat is immune to *U. occulta* and rye to *U. tritici.* One variety of wheat showed slight susceptibility to *U. agropyri,* and the smut obtained was easily propagated on the same wheat variety. These results led to the suggestion that the flag smut of wheat in the United States may have arisen as strains of *U. agropyri,* instead of having been introduced from abroad (30).

OTHER SMUTS

Grass smuts. In 1935, four physiologic races of *Ustilago striaeformis,* each restricted to a single genus of grass, were noted. Under the name of *U. clintoniana,* another closely related smut on *Dactylis glomerata* was recognized as a distinct species (18). A specialized race of *U. striaeformis* on the genera *Agropyron* and *Elymus,* which is apparently seed-borne, has been recorded. High percentages of infection were obtained on several species of *Agropyron, Elymus, Hordeum* and *Sitanion,* some species of which are recorded as new hosts for the smut (26).

Host specialization of the head smuts of grasses has been investigated. These smuts have been described under several specific names, each limited in its host range. It is now recorded that *Ustilago bullata* includes *U. bromivora* and *U. lorentziana.* Collections of the smut from various grasses were used in inoculation experiments, and eight physiologic races were differentiated (27).

Sorghum and millet smuts. Studies made in China in 1937 (173) suggest the existence of two strains or races of the millet

smut, *Ustilago crameri,* and in 1944 six distinct races were differentiated (164).

Very few additions have been made to the records of specialization in the loose (*Sphacelotheca cruenta*) and covered (*S. sorghi*) smuts of sorghum. Previously, two races of loose smut and five of the kernel smut had been described (88, 90). Minor morphological differences in a physiologic race of loose smut have been noted (108). A new race of covered smut has been isolated from cultures from sporidia, and is characterized by its ability to infect hybrids from a cross of Hegari-Kafir, highly resistant to the hitherto known races of this smut (149).

POWDERY MILDEWS

In 1937, two additional races of the powdery mildew of barley were described. These races were unable to infect various wild species of the genus *Hordeum* (142). In 1944, three additional races were isolated in Canada, making a total number of seven found in that area (13).

In 1938, additional physiologic races of wheat powdery mildew were identified in Germany (111, 115). Three physiologic races have been described from Argentina, and it was noted that practically all varieties of wheat extensively cultivated in Argentina are very susceptible. However, a few foreign varieties are resistant and may prove useful in breeding work. The various species of wheat—*Triticum durum, T. polonicum, T. turgidum, T. spelta, T. macha* and *T. compactum*—are susceptible. There are both resistant and susceptible varieties in *T. monococcum, T. aestivum* and *T. dicoccum. T. timopheevi* is resistant (159).

A large number of collections of species and varieties of grass have been tested with mildew from eight different grass hosts, and it was found that all of the cultures possessed a wide capacity of infection, attacking species of many genera. The powdery mildew from wheat and barley infected certain grass species, and a culture isolated from *Agropyron repens* infected a barley variety. The point is stressed that there may be a wide range of hosts, as well as sharp specialization within the grass mildew (41).

In 1945, five races were differentiated on species of *Poa,* and evidence of specialization of the oat mildew was obtained (42, 43).

The powdery mildew *Erysiphe cichoracearum,* in 1925 and later,

was very destructive on cantaloupes in the Imperial Valley of California. Breeding mildew-resistant melons was undertaken, and a marked degree of success was obtained. In 1938, however, it was found that certain of the new resistant varieties became severely infected, and tests showed that a new physiologic race of the powdery mildew had appeared, severely attacking practically all the commercial varieties. This necessitated an attempt to develop types resistant to the new race (66).

Specialization has been found in other species of mildew. Studies have been made with the powdery mildew of phlox (*Erysiphe cichoracearum*) and some evidence of the occurrence of physiologic races secured (85). The existence of six physiologic races of *Sphaerotheca fuliginea* was demonstrated, these also being separated on the basis of the average length of the conidia (49). Studies in Russia on the same powdery mildew established three distinct races, one limited to *Calendula,* the second to *Bidens* and the third to *Taraxacum* (112).

OTHER PATHOGENS

Many other groups of pathogenic fungi have been investigated, and physiologic specialization has been observed to be of wide occurrence. Physiologic races have been found in fungi belonging to very diverse groups, such as *Bremia lactucae* (64, 116), *Cercospora oryzae* (67, 113), *Cladosporium fulvum* (75), species of *Fusarium* (144), *Ophiobolus miyabeanus* (143), *Phytophthora infestans* (8, 77, 103), *Pseudoperonospora humuli* (55) and *Rhynchosporium secalis* (12).

SUMMARY

Thus the investigations of the past ten years have resulted in the differentiation of many new races among a wide range of plant pathogens. The races may have a very narrow range of host plants, or they may occur on species belonging to different genera.

Some investigations have revealed minor morphological differences between many strains or varieties of pathogens, so that there is no sharp line of distinction between some of these and races, which can be recognized only by experimental tests.

The powdery mildews of peach and rose, *Sphaerotheca pannosa,* seem to differ both morphologically and pathologically, the mildew from one host not infecting the other (172). Morphological varieties have been found in *Tilletia laevis* and *T. tritici* (59, 122).

Further, problems have been raised in connection with the utilization of suitable test varieties. In the crown rust of oats, Straib (134) differentiated many more races by the use of additional host varieties, as compared with the results obtained using the set originally developed by Murphy (92). Kingsolver and Murphy (73) obtained similar results in their experiments. Hassebrauk (54), on the basis of his work with the leaf rust of wheat, suggested that the differential hosts appropriate for one country may not be suitable for another. Rashevskaya and Barmenkoff (101) also found that the so-called standard varieties used for differentiation were inadequate for distinguishing physiologic races of this rust in collections made in Russia. Honecker (60, 61) found the varieties of barley used by Mains and Dietz (86) to be unsatisfactory for differentiation of barley mildew races in Germany.

Based on physiologic tests, many pathogenic fungi usually recognized as distinct species, seem to be distinguished best as physiologic races. There would appear to be no sharp lines of separation between *Urocystis agropyri, U. occulta* and *U. tritici* (30). The head smuts of grasses usually listed under different specific names certainly are closely related (27).

Another problem is raised in some investigations on *Phytophthora infestans*. Black (8) has recorded two strains, one being more virulent on certain hybrids than the other. Lehmann (77, 78) has suggested the existence of eight biotypes or races, recording great differences in their virulence as well as in their distribution. Reddick (102, 103) has discussed the question of the origin of *P. infestans* and some of the problems in breeding for resistance to this organism, having recorded four physiologic races as being known in North America. He also suggested that the virulence of the fungus is enhanced by passage through a resistant host, and thus it might be possible that an almost infinite range of races would develop if suitable intermediate host varieties were produced.

It is evident from many of the investigations reported that there is need for further standardization of methods and the control of environal conditions in relation to both host and pathogen.

Many pathogens may be grown in culture in the laboratory, and isolates may show distinct racial differences. Christensen and Graham (14) distinguished more than 125 cultural races of *Helminthosporium gramineum* Rabh. Utter (148), in studies on cul-

tures of a race of loose smut and another of covered smut of oats, was able to differentiate many distinct types. Further, from hybrids between the two species, he isolated new races combining the morphological characters of the two smuts with new capacities for infecting oat varieties.

In an important and wide-spread crop such as wheat, great changes have occurred in the distribution of specialized races. Race 56 of *Puccinia graminis,* which was first found in 1928, has rapidly increased in the United States and Canada.

The races in one country may differ decidedly from those in another, and varieties of crop plants may escape infection, due to the absence of races which attack them. The importation of new races from one locality to another may result in serious damage to varieties which were considered resistant.

PHYSIOLOGIC RACES IN CEREAL RUSTS

Fungus	Authority		Year	Country	No. of races
Puccinia anomala Rostr.	Straib	(133)	1937	Europe	14
	D'Oliveira	(20)	1939	England, Portugal, Spain	11
	D'Oliveira	(20)	1939	World	30
Puccinia coronata (Pers.) Corda	Frenzel	(33)	1930	Germany	33
	Murphy	(92)	1933	North America (1927–1932)	33
	Brown	(11)	1937	England and Portugal	4
	Straib	(134)	1937	Germany	139
	Waterhouse	(166)	1938	Australia	5
	Vallega	(151)	1940	Argentina	4
Puccinia glumarum (Schm.) E. & H.	Straib	(131)	1937	South America	4
	Straib	(132)	1937	Germany	38
	Straib	(136)	1939	Alps Region, and Bulgaria, Japan	14
	Mehta	(87)	1940	India	8
	Fang	(23)	1944	China	9
Puccinia graminis Pers. *avenae* E. & H.	Verwoerd	(161)	1931	South Africa	2
	Levine and Smith	(79)	1937	U. S.	5
	Waterhouse	(166)	1938	Australia	6
	Hassebrauk	(53)	1939	Germany	7
	Kummer	(74)	1939	Germany	1
	Newton *et al.*	(94)	1940	Canada	11
	Vallega	(158)	1943	Argentina	2
	Newton and Johnson	(93)	1944	Canada	13

Fungus	Authority		Year	Country	No. of races
Puccinia graminis Pers. *secalis* Eriks.	Verwoerd	(161)	1931	South Africa	2
	Cotter and Levine	(17)	1932	U. S.	14
	Stakman	(124)	1935	U. S.	14
	Kummer	(74)	1939	Germany	2
Puccinia graminis Pers. *tritici* E. & H.	Verwoerd	(161)	1931	South Africa	8
	Dodoff	(19)	1934	Bulgaria	8
	Stakman *et al.*	(126)	1934	World	127
	Tu	(145)	1934	China	6
	Sibilia	(119)	1936	Italy	2
	Hassebrauk	(50)	1937	Germany and Southern Europe	7
	Sibilia	(120)	1939	Italian East Africa	17
	Waterhouse	(166)	1938	Australia and New Zealand	9
	Garbowski	(34)	1939	Poland	7
	Hassebrauk	(53)	1939	Germany	11
	Kummer	(74)	1939	Germany	5
	D'Oliveira and DeSousa	(21)	1940	Portugal	6
	Vallega	(150)	1940	South America	7
	Garcia-Rada *et al.*	(35)	1942	Peru	1
Puccinia triticina Eriks.	Mains	(81)	1933	World	53
	Asuyama	(5)	1935	Japan	2
	Goeschele	(39)	1936	Odessa	5
	Humphrey *et al.*	(62)	1936	World	86
	Rashevskaya and Barmenkoff	(101)	1936	U.S.S.R.	13
	Roberts	(107)	1936	England and Portugal	10
	Hassebrauk	(51)	1937	Europe	11
	Petrusheva	(97)	1937	Leningrad	4
	Ralski	(100)	1937	Poland	5
	Vohl	(163)	1938	Europe	16
	Sibilia	(121)	1939	Libya	1
	Sibilia	(121)	1939	Abyssinia	3
	Mehta	(87)	1940	India	6
	Vallega	(152)	1941	Brazil	3
	Yarkina	(169)	1941	Saratoff	4
	Johnston *et al.*	(71)	1942	World	129
	Hassebrauk	(54)	1940	Europe	45
	Vallega	(155)	1942	Argentina	9

PHYSIOLOGIC RACES IN OTHER RUSTS

Fungus	Authority		Year	Country	No. of races
Gymnosporangium clavipes C. & P.	Miller	(91)	1939	U. S.	2
Melampsora lini (Pers.) Lév.	Flor	(31)	1935	U. S. & Canada	14
	Straib	(135)	1939	Europe	4
	Flor	(32)	1940	U. S. & So. America	24
	Waterhouse	(167)	1943	Australia	6
	Vallega	(156)	1942	Argentina	5
Puccinia antirrhini D. & H.	Yarwood	(171)	1937	Calif.	2
Puccinia helianthi Schw.	Brown	(10)	1936	Canada	4
Puccinia iridis (DC.) Wallr.	Mains	(83)	1934	U. S.	2
	Mains	(84)	1938	U. S.	3
Puccinia sorghi Schw.	Mains	(82)	1934	U. S.	2
	Vallega	(154)	1942	Argentina	2
Uromyces graminis (Niessl) Diet.	Gäumann	(37)	1940	Germany	3
Uromyces dactylidis Otth.	Gäumann	(38)	1941	Germany	6
Uromyces festucae Syd.	Gäumann	(38)	1941	Germany	3
Uromyces poae Rabh.	Gäumann	(38)	1941	Germany	8
Uromyces phaseoli (Pers.) Wint.	Harter *et al.*	(47)	1935	Calif. & Wash., D. C.	2
	Parris and Matsuura	(96)	1941	Hawaii	2
	Dundas and Scott	(22)	1939	Fla. & Wash.	2
	Harter	(46)	1939	U. S. & Hawaii	13
	Harter and Zaumeyer	(48)	1941	U. S.	20

PHYSIOLOGIC RACES IN CEREAL SMUTS

Fungus	Authority		Year	Country	No. of races
Sphacelotheca cruenta (Kühn) Potter	Melchers	(88)	1933	U. S.	2
	Melchers	(89)	1940	U. S.	2
Sphacelotheca sorghi (Link) Clint.	Melchers *et al.*	(90)	1932	U. S.	5
	Melchers	(89)	1940	U. S.	3

Fungus	Authority		Year	Country	No. of races	
					Laev.	*Trit.*
Tilletia laevis Kühn, and *T. tritici* (Bjerk.) Wint.	Nieves	(95)	1935	Argentina	4	9
	Holton and Heald	(57)	1936	Washington	..	2
	Spangenberg and Gutner	(122)	1936	Russia	3	5
	Rodenhiser and Holton	(109)	1937	U. S.	8	11
	Churchward	(15)	1938	Australia	4	9
	Savulescu and Sandu-Ville	(114)	1938	Rumania	5	5
	Holton and Rodenhiser	(59)	1942	U. S.	10	14
					Ave.	*Lev.*
Ustilago avenae (Pers.) Jens., and *U. levis* (K. & S.) Magn.	Radulescu	(99)	1935	Rumania	4	..
	Vaughan	(160)	1938	U. S.	1	..
	Reed	(105)	1940	World	29	14
Ustilago hordei (Pers.) K. & S.	Tapke	(138)	1937	U. S.	8	
	Yu	(174)	1940	China	5	
	Yu and Fang	(175)	1945	China	9	
Ustilago nigra Tapke	Tapke	(137)	1936	U. S.	2	
	Josephson	(72)	1942	U. S.	8	
	Tapke	(139)	1943	U. S.	7	
Ustilago nuda (Jens.) K. & S.	Thren	(141)	1941	Europe	2	
Ustilago tritici (Pers.) Rostr.	Radulescu	(98)	1935	Rumania and Czechoslovakia	4	
	Hanna	(40)	1937	Manitoba	4	

PHYSIOLOGIC RACES IN POWDERY MILDEWS

Fungus	Authority		Year	Country	No. of races
Erysiphe cichoracearum DC.	Jagger *et al.*	(66)	1938	Calif.	2
Erysiphe graminis (DC.) *avenae*	Hardison	(43)	1945	U. S.	2
Erysiphe graminis (DC.) *hordei*	Mains and Dietz	(86)	1930	U. S.	5
	Tidd	(142)	1937	U. S.	2
	Honecker	(60, 61)	1937, 1938	Germany	9
	Cherewick	(13)	1944	U. S. & Canada	7
Erysiphe graminis (DC.) *poae*	Hardison	(42)	1945	U. S.	5
Erysiphe graminis	Mains	(80)	1933	U. S.	2

Fungus	Authority		Year	Country	No. of races
(DC.) *tritici*	Rosenstiel	(111)	1938	Germany	6
	Schlichtling	(115)	1939	Germany	6
	Vallega and Cenoz	(159)	1941	South America	3
Erysiphe polygoni DC.	Yarwood	(170)	1936	California	2
Sphaerotheca humuli (DC.) Burr.	Hashioka	(49)	1938	Japan	6
fuliginea (Schl.) Salm.	Rud	(112)	1938	Russia	3
Sphaerotheca pannosa (Wallr.) Lév.	Yarwood	(172)	1939	Calif.	2

PHYSIOLOGIC RACES IN OTHER PATHOGENS

Fungus	Authority		Year	Country	No. of races
Bremia lactucae Regel	Jagger and Chandler	(64)	1933	U. S.	5
	Schultz and Röder	(116)	1938	Europe	2
Cercospora oryzae Miy.	Ryker	(113)	1943	U. S.	3
	Jodon *et al.*	(67)	1944	U. S.	4
Cladosporium fulvum Cooke	Langford	(75)	1937	Canada	4
Fusarium avenaceum (Fr.) Sacc.	Tu	(144)	1930	U. S.	2
Fusarium culmorum (W. G. S.) Sacc.	Tu	(144)	1930	U. S.	3
Fusarium gramineum Schwabe	Tu	(144)	1930	U. S.	3
Helminthosporium gramineum Rabh.	Arny	(4)	1945	U. S.	2
Helminthosporium maydis Nisik. & Mke.	Ullstrup	(146)	1941	U. S.	2
Ophiobolus miyabeanus Ito and Kuribayashi	Tochinai and Sakamoto	(143)	1937	Japan	10
Phytophthora infestans de Bary	Lehmann	(77)	1937	Germany	8
	Reddick	(103)	1940	North America	4
	Black	(8)	1943	Scotland	2
Pseudoperonospora humuli (Miy. & Tak.) Wils.	Hoerner	(55)	1940	U. S.	4
Rhynchosporium secalis (Ond.) Davis	Caldwell	(12)	1937	U. S.	6
Sclerospora graminicola (Sacc.) Schroet	Uppal and Desai	(147)	1932	India	2

Fungus	Authority		Year	Country	No. of races
Selenophoma bromigena (Sacc.) Sprague & Johnson	Allison	(3)	1945	U. S.	2
Septoria tritici Desm.	Sprague	(123)	1934	U. S.	1
Synchytrium endobioticum (Schilb.) Perc.	Braun	(9)	1942	Europe	2
Thielaviopsis basicola (B. & Br.) Zopf.	Allison	(2)	1938	U. S.	4

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RESPIRATION. II[1]

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INTRODUCTION

When the subject of respiration was reviewed in this journal 11 years ago it was stated that by respiration was understood those processes occurring in every living cell which involve a release of chemical energy utilised partly in building up compounds of higher energy content and in other vital processes. The writer has since pointed out (65) that there are many organs, such as storage tissues and mature fruit, which do not grow but which, nevertheless, respire actively, although no synthesis of compounds of higher energy content nor any other process needing a supply of energy appears to take place in them, and that it would appear that respiration is concerned in some way in the very maintenance of tissues in a living condition. The energy aspect of respiration has been discussed at some length by Wohl and James (76) who divide the energy released in respiration into that required for growth and that required for mere maintenance. As regards the latter they conclude that nearly the whole of the energy released by plants in what they call the mature phase escapes as heat which is useless to the plant. During growth only the energy which is fixed by synthetic reactions is used by the plant, the rest of the energy released in respiration being lost as heat.

In dealing with the energy relations of those reactions which are supposed to occur in respiration they point out that metabolic reactions are of two kinds, those which occur spontaneously and involve a decrease in free energy, and others, which may be called "driven reactions", which are possible only if supplied with energy. This energy supply may come from spontaneous reactions, and Wohl and James discuss a number of metabolic reactions in which linkage of spontaneous and driven reactions may occur.

These writers also discuss the energy relations of anaerobic respiration. Although the decrease of free energy in the reaction $C_6H_{12}O_6 = 2\ C_2H_5OH + 2\ CO_2$ is 71.9 cal. per mol., they conclude that all the evidence indicates that in general this energy is useless to the plant.

[1] Supplement to article in The Botanical Review 1: 249–268. 1935.

In commenting on the discussion by Wohl and James, Boswell (12) points out that of the two kinds of reaction which respiration involves, hydrolytic and oxidative, it is the latter which is the principal source of energy. These reactions are regarded as forming a step-by-step series whereby hydrogen (or electrons) are transferred between the substrate and oxygen. Each reaction in the series has a characteristic free-energy change which determines its position in the sequence of reactions, since it also determines what reaction it may drive or by what reaction it may be driven. There thus results an orderly progress of the respiratory mechanism, the heat liberated being the final manifestation of the free-energy changes of all these successive reactions. (See also 16.)

NOMENCLATURE

The last decade has produced a number of changes in nomenclature. Thus there is a growing tendency to denote the production of carbon dioxide and alcohol by plants in absence of oxygen as fermentation instead of anaerobic respiration, on the assumption that the process is identical with fermentation by yeast. The chief argument in favour of this change is that the process has been shown to occur in presence, as well as in absence, of oxygen, so that the word anaerobic is definitely misleading. On the other hand, there are tissues, such as those of potato tuber, in which, although carbon dioxide is produced in absence of oxygen, very little alcohol, or even none, is recognisable. To the writer it would therefore appear that, apart from its brevity, the term "fermentation" has little advantage over the term "anaerobic respiration" to denote the production of carbon dioxide in absence of oxygen.

It is generally agreed that in the early stages of both aerobic and anaerobic respiration, hexose is broken down to compounds containing three carbon atoms. It has been customary to speak of this breakdown as "glycolysis", but Turner (71) has pointed out that the formation of lactic acid from glycogen, hexoses and methyl glyoxal which occurs in animal tissues and yeast under certain conditions, is widely known as "glycolysis", whereas in plant respiration lactic acid is certainly not formed in any appreciable amount. To avoid confusion in the use of the term "glycolysis" he has therefore suggested a new term, "triosis", to express the production from a hexose, or some phosphorylated product of a hexose, of an unspecified 3-carbon compound other than lactic acid.

Another term which has come much into use during the last ten years is "Pasteur effect" or "Pasteur reaction". This refers to a phenomenon which is frequently, though not always, observed when tissue is transferred from aerobic to anaerobic conditions, or *vice versa*. If in presence of oxygen the products of respiration are carbon dioxide and water and in absence of oxygen they are carbon dioxide and ethyl alcohol, then if the rate of hexose consumption is the same under both sets of conditions, the ratio of carbon dioxide produced under aerobic conditions to that produced under anaerobic conditions will be 3, as an inspection of the equations

$$C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O$$
$$C_6H_{12}O_6 \qquad = 2\ CO_2 + 2\ C_2H_5OH$$

at once makes clear. But in many tissues a ratio of less than 3, and sometimes very much less, has been observed, which means that the presence of oxygen lowers the rate at which sugar breakdown occurs in its absence. This is the Pasteur effect.

One other expression may be noted, namely, "extinction point", a term which appears to be due to F. F. Blackman. In low oxygen concentrations respiratory quotients are observed which are greater than unity and which indicate that both aerobic and anaerobic respiration (fermentation or zymasis) are taking place. As the oxygen concentration is increased the respiratory quotient falls until it reaches a constant value in the neighbourhood of unity, indicating the extinction of the anaerobic component of the respiration. This oxygen concentration is called the "extinction point".

METHODS

Of recent years manometric methods, with which the names of Barcroft, Warburg, Thunberg and Fenn are associated, have become increasingly popular with workers on plant respiration. These methods, with a full account of the precautions necessary in their use, are described in a book (*22*) which will be found invaluable to botanists using the manometric technique. A paper by Brown (18) may also be consulted.

In the writer's earlier account of respiration in this journal reference was made to the development by Stiles and Leach of the katharometer for the measurement of respiration. In their apparatus the respiration vessel was closed, an arrangement which is

often quite satisfactory but which may not be suitable for some researches on respiration. Now a modification of the katharometer for use in an experimental arrangement in which a continuous current of gas passes over the plant material, has been described (6, 7). The actual experimental work carried out was limited to measurements of photosynthesis, but the instrument could be equally well used for work on respiration.

The change in electrical conductivity of a solution of barium hydroxide as a result of absorption of carbon dioxide was used many years ago by Spoehr and McGee as a basis for the measurement of plant respiration. More recently apparatus in which the same principle has been used has been described (57). In it a specially designed conductivity cell is used with an alternating current Wheatstone's bridge in which the alternating current is derived from an oscillating thermionic valve with valve amplification. By means of this apparatus a quantity as small as 0.11 μg. of carbon dioxide could be determined.

A similar apparatus has been described elsewhere (21) in which sodium hydroxide replaces barium hydroxide and in which, instead of measuring the conductivity by balancing the resistances in the arms of the Wheatstone's bridge as the resistance of the sodium hydroxide solution changes with absorption of carbon dioxide, the resistances in the other three arms are kept constant, and the current flowing through the galvanometer is used as a measure of the conductivity. The apparatus is made self-registering by using a recording ammeter for the galvanometer.

The method has been developed more recently by others (49). Their arrangement comprises an oscillator, amplifier and vacuum tube voltmeter working in conjunction with a specially designed absorption tube in which sodium hydroxide is used to absorb the respiratory carbon dioxide. The change in resistance of the solution in the absorption tube is not determined by balancing resistances in a Wheatstone's bridge, but by measuring, by means of a diode vacuum tube voltmeter, the change in the potential across the cell when a constant alternating current of frequency of about 1,000 cycles per second flows through the cell. The circuit is so arranged that the deflection of the associated galvanometer is almost directly proportional to the change in resistance of the solution in the absorption tube. By substituting a strip of bromide

paper for the galvanometer scale and introducing into the galvanometer lamp circuit a clockwork time switch that closes the circuit for a few seconds every hour, the apparatus can be made self-recording. With the apparatus used by the designers it was possible to measure quantities of carbon dioxide down to 10 μg., but the sensitivity could be increased by the use of a more sensitive galvanometer.

Lastly, mention should be made of the work of those (58) who have used the principle of the polarograph for the determination of small quantities of dissolved oxygen and shown how it can be used for measuring the respiration of erythrocytes and some other animal tissues. The method has been extended to the measurement of the respiration of plant material by others (23) who, by its use, have measured the respiration rate of a single oat coleoptile.

RESPIRATION DRIFTS

A number of researches have been described in which data on the drift of respiratory activity with time have been obtained, *e.g.*, in Conference pears during storage at 10° C. (40). After gathering, the respiration rate falls until at maturity the rate begins to increase. This is the "climacteric rise" which may reach two or three times the preceding rate. The climacteric rise is followed by a further rise accompanying a breakdown of the flesh of the fruit, and this again is followed by a final fall in respiration rate as the tissue dies. This respiratory behaviour of pears is similar to that previously described by Kidd and West for apples. The respiratory activity of the fruit on the tree is very closely correlated with the protein and acid contents. During storage the climacteric rise appears to be accompanied by rises in the ratio of protein to alcohol-soluble nitrogen and in sucrose content. Determinations of various nitrogen fractions and carbohydrates throughout the ripening and storage period suggest that respiration involves a utilisation of glucose throughout, and of fructose as well after the climacteric phase. They further suggest that glucose units are formed from the alcohol-insoluble residue which includes starch and that fructose units are formed from glucose units during hydrolysis of starch.

The climacteric rise in McIntosh apples has twice been observed (59, 41). In the latter is a detailed study of the respiration of these apples throughout their ontogeny from a fortnight after fruit setting

until the death in storage of all apples picked later. For this purpose samples of apples were taken at different dates and their respiration followed until breakdown of the apple was evident. The initial respiration rates of these samples may be regarded as those of apples on the tree and in storage, and indicate a continuous downward drift from the middle of June to just before the usual time of harvesting when the climacteric rise occurs. This is followed by a slow fall in respiratory activity until death occurs. In some of the respiration drifts of the different samples pre-climacteric and post-climacteric rises were also observed.

Krotkov considers the continuous pre-climacteric fall in respiratory activity to be due to a continuous fall in the concentration of the respiratory substrate. When this has reached so low a value that the respiration rate is insufficient to provide sufficient energy for the maintenance of the protoplasmic organisation, either a fresh source of the substrate or an altogether new substrate becomes available, and the climacteric rise results. As the concentration of substrate again falls, so does the respiration rate until death occurs, when the rate becomes too low to provide the energy for protoplasmic maintenance. It is to be observed that the minimum pre-climacteric rate and the final rate just before death are about the same.

The drift of respiration in developing Jonathan apples has been followed (62), using the Barcroft manometric technique, over a period from 23 May to 26 July. Up to 22 June whole fruits were used and after that date a sector of the apple cut out vertically from stem to calyx was used. The effect of cutting was to increase the respiration rate by about 63%. Shaw found the respiratory activity increased rapidly from an average of 28.56 µl. of oxygen per hour per gram of fresh weight on 23 May to a maximum of 71.88 µl. on 30 May, after which the rate fell rapidly to an average of 12.66 on 22 June, and then very slowly. The period of high respiratory activity is that immediately following fruit setting when there is rapid cell division throughout the flesh of the fruit. The respiratory quotient also underwent marked changes, falling from 0.84 to 0.45 during the period 23 May to 22 June and then gradually rising until on 26 July it reached the value of 0.92. The low values may be the result of the utilisation of oxygen in the production of malic acid.

The drift of respiratory activity in a number of vegetables in storage has been followed (60). The material included fruits (peppers, tomatoes, cucumbers), seeds (peas and beans), roots (carrot), tubers (potatoes) and vegetative parts (asparagus, spinach and lettuce). The course of respiration was followed at three temperatures (0.5°, 10°, 24°) for periods up to 60 days. At all the temperatures the respiration rate fell gradually with time, the decline being most rapid during the first few days and more rapid the higher the temperature. This fall in respiratory activity might be due to lowering of the immediate substrate concentration or to other physiological processes associated with ageing of the cells.

Platenius also determined the respiratory quotients for the various vegetables during their storage. For most of them the quotient was in the neighbourhood of unity, although there was a tendency for the quotient to become slightly lower in prolonged storage. Notably lower quotients were, however, observed with asparagus, spinach and potatoes. Determinations of the sugar content of asparagus at harvesting and after three days in storage showed that the total sugar lost could account for only half the carbon lost in respiration. Since asparagus contains no starch and only a minimum quantity of fat, it must be concluded that protein is utilised to a considerable extent in the respiration of this plant. Since protein gives a respiratory quotient of 0.80 to 0.82, the low values of the quotient found for asparagus can be attributed to a utilisation of protein as well as of sugar for respiration. The same explanation can be suggested for the low R.Q. of spinach, but cannot be valid for potatoes where quotients as low as 0.45 to 0.66 were observed with tubers stored at 0.5° C. There is a possibility, described by Platenius as remote, that part of the oxygen absorbed is used in the formation of organic acids.

The respiration drift in a number of whole storage organs, potato and artichoke tubers, and carrot roots, at 25° C. has been followed (20). Typically, the drift exhibits two phases, first a fairly rapid rise to a maximum and then a very slow fall, sometimes to an approximately constant level. The initial rise is attributed to the change from the lower temperature of storage to the higher temperature of experimentation, one of the factors inducing the increase being evolution of carbon dioxide released from solution

as a result of the rise in temperature. Some evidence of a seasonal drift was obtained, the respiratory rate of potatoes appearing to fall as the storage season advanced, and rising again later in the season.

This high initial output of carbon dioxide has also been observed (1) in a number of vegetables, including potatoes, on transference from cool storage to a temperature of 22° C. The workers are of the opinion that the high initial carbon dioxide output in these circumstances was not due to any release of carbon dioxide dissolved in the sap, for if this were so the same burst of carbon dioxide should occur in nitrogen, but it does not. They agree, however, that the initial rise is a definite change-of-temperature effect. In general, the respiration drifts of the organs examined by Appleman and Smith were similar to those of the organs examined by Choudhury, the initial high rate being followed by a fall to a lower rate which either remained constant or fell continuously till the end of the experimental period.

A detailed examination of the drift of respiration in barley germinating in the dark has been made (35). With whole grains the workers distinguish five phases in the respiration drift. First there is a phase of rapidly increasing respiration rate corresponding to free embryonic development, lasting at 21° C. for two days. This is followed by a phase in which the acceleration in the rate slows down. By the seventh day the respiration rate reaches its maximum after which the rate falls with increasing rapidity for a further five days (phase 3). The second and third phases correspond, respectively, to the mobilisation of endospermic carbohydrate reserves and to their exhaustion. In the fourth phase the fall in respiration rate ceases to be rapid but proceeds slowly to a minimum; this is held to correspond to the breakdown of other materials, probably protein. On the twenty-second day from the beginning the respiration begins to rise on account of the activity of micro-organisms. As far as the early part of the period is concerned, these results agree with the earlier ones of Barnell (10).

The course of respiration in germinating wheat grains of a number of varieties has been followed and its relationship to water absorption examined (46). Three respiratory stages during germination can be distinguished. The first phase is characterised by a slowly increasing rate which begins soon after the grain is

brought into contact with water. This is succeeded by a phase marked by a rapid increase in rate followed by a decrease. Later the rate again increases and follows an approximately uniformly rising course characterising the final stages of germination. The slow initial rise in the respiration rate does not appear to be related to water content and so does not appear to be due to water shortage but must be attributed to some factor other than deficiency of water.

In a second paper Leach (47) describes the effect of age and kernel size on the course of respiration of germinating wheat. No significant effect appeared in the respiratory activity as a consequence of the different storage periods, 6, 18 and 30 months. Kernel size, however, was found to have a very definite effect on the rate of respiration expressed in terms of carbon dioxide output per unit weight of grain, the rate being higher in small kernels than large ones. From this it is to be concluded that the respiratory activity is mainly in the embryo and that the endosperm contributes a relatively small proportion of the total respiratory activity of the grain.

EFFECT OF EXTERNAL CONDITIONS ON RESPIRATION

Temperature. Gane (24) has measured the respiration of unripe bananas at various temperatures between 12.5° C. and 32° C. Over this range he found the value of the respiration was given by the equation $\log R = 0.843 + 0.0348\ t$ where R is the respiration rate and t the temperature. This gives a temperature coefficient (Q_{10}) of 2.23, a value similar to that previously obtained by other workers for a variety of tissues.

Water content. The relationship of respiratory activity to the water content of wheat grain has been examined by Shirk and Appleman (64). Samples of Minhardi wheat grains were soaked in distilled water at 28° C. for different periods of time varying from 0.25 to 5.75 hours, and the total water, freezable water and rate of oxygen absorption determined in each sample of grain. It was found that the respiration rate ran very parallel with the content of freezable water. According to Shirk and Appleman the rate of respiration of grain following imbibition of water decreased rapidly for five to seven hours and then more slowly for almost 20 hours. Throughout this time an equilibrium between bound and

free water was being established, the freezable water-content continuously falling. Throughout the period the fall in respiration rate was closely parallel to the fall in the freezable water-content. Later work by Shirk (63) on developing wheat and rye grains confirmed the parallelism between respiration and freezable water.

Oxygen concentration. Results so far obtained on the effect of oxygen concentration on the respiration rate have varied very considerably with different tissues. Choudhury (20) has measured the respiration of whole potato tubers, whole artichoke tubers and whole carrot roots in atmospheres containing quantities of oxygen ranging from 3.5% to 100%. The effect of oxygen concentration was different with each of these organs. With potato variations in oxygen concentration between 6.2% and 100% had no appreciable effect on the carbon dioxide output, but with carrot low concentrations of oxygen (3.5%) in one root caused a lowering in the respiration rate below that occurring in air, in another root it rose above the level in air; in both there was a tendency for the rate characteristic of air to be regained. Concentrations of oxygen above that of this gas in air brought about increases in respiration, the rate being higher the more concentrated the oxygen, so that the highest rate was observed in 100% oxygen. With artichoke the respiration in pure oxygen was the same as in air, but in three concentrations of oxygen below that of this gas in air, 10.7%, 6.7% and 3.5%, the respiration was lowered, the decrease being greater the lower the oxygen concentration.

The difference in the results obtained with these three organs is probably due to two factors, the different rates of respiration in the different tissues and their different capacities for anaerobic respiration. In the potato the observed respiration rates in air were only about a quarter of those found for carrot and a third of those found for artichoke. With potato the lowest concentration of oxygen employed, 6.2%, was sufficient to effect the oxidation of the respiratory substrate at the same rate as that occurring in air; that is, some factor other than oxygen concentration was the limiting one. In artichoke, on the other hand, when the concentration of oxygen fell below that of the gas in air, oxygen concentration did become a factor limiting the rate of oxidation. With carrot it would seem likely that oxygen limited the rate of respiration at still higher tensions, but with this organ the matter is complicated

by the incidence of anaerobic respiration (fermentation), which is shown by a rate of carbon dioxide output in nitrogen which may exceed that occurring in air. In artichoke the rate of carbon dioxide output in nitrogen fell decidedly below, and in potato very much below, that taking place in air.

Marsh and Goddard (55) found that the oxygen absorption by thin slices of carrot in concentrations of oxygen from 5% downwards fell progressively with lowering of the oxygen concentration, but that the carbon dioxide output increased so that the respiratory quotient rose from 0.82 in 5% oxygen to 3.5 in 1% oxygen. This is again explained as due to the incidence of fermentation in low oxygen concentrations, and from the data of oxygen absorption and carbon dioxide evolution Marsh and Goddard calculate that in 5% oxygen true aerobic respiration is reduced to 70% to 75% of that occurring in air, and that in 1% oxygen it is reduced to 20% or less.

An observation by duBuy and Olson (23) on the effect of oxygen concentration on the respiration of oat coleoptile submerged in a nutrient solution may be noted here. The fall in oxygen concentration of the solution was determined polarographically, and it was found that with falling oxygen concentration the respiration rate remained constant until a certain low concentration of oxygen was reached, when the respiration rate suddenly fell to half its previous value above this concentration. With young coleoptiles this critical concentration was about 0.45 volume per cent. and for old coleoptiles about 0.25 volume per cent. The significance of this finding is discussed in a later section of this review.

In an investigation which had for its main object the ascertaining of the physiological basis for the different conditions under which wheat and rice flourish, Taylor (66) has examined among other things the effect of different oxygen tensions on the respiration of seedlings of these two species. His results indicate that with both wheat and rice there is a progressive lowering of oxygen absorption as the oxygen concentration is reduced from that of air to zero. As regards carbon dioxide output, on the other hand, whereas in wheat it also falls with lowered oxygen concentration, in rice it appears to increase with decrease in oxygen concentration, and in pure nitrogen the rate of evolution appears to be about 50% higher than in air. These results suggest that the superior capacity of rice over wheat for growth under conditions of poor aeration is related to its ability to respire under anaerobic conditions.

Experiments in which the respiration of rhizomes and leaves of a number of semi-submerged plants were measured in different oxygen concentrations, are referred to later.

Carbon dioxide concentration. A high concentration of carbon dioxide was shown by Kidd many years ago to have a retarding effect on both oxygen uptake and carbon dioxide output by germinating mustard. Livingston and Franck (50), however, in an investigation concerned mainly with photosynthesis, have found this is not always so in leaves of *Hydrangea otaksa.* In leaves taken from the plant in December the respiration rate was about the same in air, in 5% carbon dioxide and in 20% carbon dioxide. Exposure of the leaves in 20% carbon dioxide to light usually depressed the rate of respiration. Sometimes the output of carbon dioxide from leaves respiring in a high concentration of carbon dioxide exceeded the intake of oxygen. This effect is reminiscent of the effect of high concentrations of carbon dioxide on the respiration of apples observed by Thomas to which he gave the name CO_2—zymasis.

Inorganic salts. The relation of inorganic salts to respiration was brought into prominence by Lundegårdh's well known theory of anion respiration based largely on that worker's observation of the parallelism between respiration rate and the amount of anion absorbed by cells from solutions of a number of salts. It is not proposed in this review, which deals primarily with respiration, to enter into the vexed and complex question of the relationships supposed to exist between respiration and accumulation of salts or their anions by various tissues, as the emphasis in the work of most recent investigators of this subject has been rather on the factors controlling salt uptake than on those controlling respiration. It is the intention of the present writer to discuss these problems elsewhere. Some recent observations, however, in which an effect of inorganic salts on respiration has been found, may be mentioned here. In particular, reference may be made to the work of Robertson (61) who examined the intake of various chlorides and the carbon dioxide evolved by thin slices of carrot root, and who concluded that all the chlorides used, namely, those of potassium, sodium, lithium, calcium and magnesium, brought about an initial increase in the rate of respiration above that of tissue maintained in distilled water. With the monovalent chlorides the high respi-

ration rate continued for some 150 to 200 hours, whereas with the divalent chlorides the rate fell off after about a day. Bennet-Clark and Bexon (11) also found that chlorides affected the rate of respiration of thin slices of red beet root. They record a progressive increase in respiration rate of this tissue when it was washed in running tap water. This development of respiratory activity is increased when the disks are kept in 0.05 N potassium chloride or calcium chloride, the effect being greater with potassium chloride than with calcium chloride.

A very marked effect of manganese chloride on the respiration of wheat roots has been observed by Lundegårdh (51). He found that the uptake of oxygen by these organs was increased from 155% to 470% by addition to the medium of 0.00005 M manganese chloride. In contrast to this, ferric chloride or ferric citrate brought about a small decline in respiration rate, the mean reduction produced by 0.00005 M ferric citrate being about 21%. Lundegårdh concluded that manganese, but not iron, was a catalyst in aerobic respiration.

An examination of the effect of ammonium chloride and a few other salts on the respiration of shoots of *Elodea canadensis* kept in the dark has been made by Jones (38). He concludes that these generally bring about an increase in the rate of respiration, but that the effect is transitory. Both in water and in salt solutions there is a fairly rapid fall in respiration to a low rate, which is related to a loss in the healthy appearance of the shoots. This behaviour is not related to limited carbohydrate supply, since the course of events is essentially the same when the shoots are pretreated with glucose or sucrose. When, however, the shoots are put in a solution of 0.05% asparagine the onset of the low respiration rate is delayed and the shoots retain their healthy appearance, while the same is the case with a solution of 0.01 M ammonium chloride + 0.05% asparagine. In the latter solution the respiration of *Elodea* also exhibits the transitory rise due to the presence of the salt. These results are interpreted by Jones as indicating that the fall in respiration rate and loss of health is related to a disturbance in the nitrogen relations of the tissue, and it is suggested that the effect of salts or prolonged darkness is to bring about an excess of protein degradation over synthesis with consequent loss of vitality.

Ethylene. Gane (24) treated bananas with ethylene in the preclimacteric phase of low respiratory activity. This treatment always brings about immediately the rise in respiration rate associated with ripening of the fruit. Gane finds that ripe bananas themselves produce ethylene and that the acceleration in ripening of the fruit produced by ethylene in a concentration of one part per million is similar to that produced by the metabolism of the ripe fruit. The conclusion is drawn that ethylene, as a normal product of metabolism during the climacteric, acts as an autocatalyst.

RESPIRATORY ACTIVITY OF DIFFERENT TISSUES

Some recent observations have been made on the different respiratory activities of different parts of the same plant or organ. Goodwin and Goddard (27) have made such observations on tissues isolated from the wood of ash and maple in early spring before opening of the buds. They find that respiration is then most active in the cambium and somewhat less active in the adjoining secondary phloem and xylem. Oxygen uptake becomes progressively less in passing from the cambium to the middle of the tree. In the heart-wood there is a low rate of oxygen absorption which is not destroyed by boiling and which is probably to be attributed to the slow oxidation of organic substances in the dead cells. This also occurs in phloem and sapwood where it contributes a little to the total oxygen consumption. After opening of the buds the respiration of cambium, phloem and sapwood of the ash is about the same as before, but the respiratory activity of the newly formed and differentiating xylem considerably exceeds that of the cambium.

Machlis (53) has measured the respiration rate of portions of barley roots cut at different distances from the root apex, and, as might be expected, found that the respiratory activity decreased with increasing distance from the apex.

RESPIRATION OF PLANTS OF VARIOUS TYPES

Semi-submerged plants. The respiration of plants possessing rhizomes which live in submerged mud presents a special problem in regard to their respiration, for their conditions of life are almost anaerobic. A special study of this problem has been made by Laing (42, 43, 44).

It may be noted at the outset that the oxygen content of the in-

ternal atmosphere in the rhizomes of these plants may be very low. Laing analysed the internal atmosphere of petioles, culms and rhizomes of a number of semi-submerged plants belonging to the following species: *Nuphar advenum, Peltandra virginica, Pontederia cordata, Typha latifolia, Sparganium eurycarpum* and *Scirpus validus*. In *Nuphar advenum*, for example, the oxygen concentration of remote parts of the rhizome can fall as low as 0.6 volume per cent., although during the afternoon of a sunny day in summer the oxygen content can rise as high as 7%. Rather similar low values were also observed in *Peltandra virginica, Sparganium eurycarpum* and *Scirpus validus*. The concentration of carbon dioxide, on the other hand, can rise to remarkably high levels, values between 10% and 20% being common, while in submerged young rhizomes of *Typha latifolia* values of 27.5% and 38% were recorded. Measurements of the respiration by a Pettenkofer method of the rhizomes and leaves of the plants mentioned above and of others growing in a similar habitat were made in which the organs were exposed to air, nitrogen and various gas mixtures containing oxygen in concentrations ranging from 0.1% to 10%. It was found that the rhizomes of all the plants examined were able to respire anaerobically in nitrogen for several days without appreciable injury. The rhizomes of *Nuphar advenum* appear to be particularly resistant to injury from anaerobic conditions. The rate of evolution of carbon dioxide was, however, somewhat lower in nitrogen than in air. On the whole, the respiration rate in the gas mixtures decreased with decrease in the oxygen concentration, although numerous exceptions to this occurred; it is not clear to what extent these exceptions are due to variations in the material used. With leaves the results were different. It was found that young leaves of *Nuphar advenum* and the leaves of *Typha latifolia* could respire anaerobically for some days, although the initial rate was not maintained over this length of time, the rate falling to about half in four days. With older leaves of *Nuphar advenum*, however, the capacity to respire anaerobically was very much less, the rate of carbon dioxide output at the end of four days being only about 6% of the original.

The general conclusion may be drawn from this work that the rhizomes of semi-submerged plants respire both aerobically and anaerobically, the extent to which one or other of these processes

predominates varying with conditions, particularly with those which induce photosynthesis in the leaves and the consequent setting up of oxygen and carbon dioxide diffusion gradients in the internal aerating system throughout the plants.

Arctic plants. Wager (75) has measured the respiration rates of a number of arctic plants at temperatures from 0° to 40° C., and has calculated the temperature coefficients (Q_{10}) for the four temperature intervals 0°–10°, 10°–20°, 20°–30° and 30°–40°. In general, the coefficient falls with rising temperature. Thus for shoots of *Cassiope tetragona* the coefficients were 3.3, 2.9, 2.2 and 1.7, over the intervals 0°–10°, 10°–20°, 20°–30° and 30°–40°, respectively. For leaves of *Antennaria alpina* the corresponding values of the temperature coefficients were found to be 4.2, 3.3, 2.0 and 1.6, and for shoots of *Saxifraga oppositifolia* 3.0, 2.7, 2.2 and 1.9. Wager made determinations on both winter and summer plants, the species used in the two sets of experiments being different. He found the average Q_{10} of the respiration rate of the summer plants was less than that of the winter plants (2.3 as against 2.7 for the 10°–20° interval). This is attributed to a higher substrate concentration in the plant cells in winter, support for which idea is found in the higher osmotic pressure of the plants in winter, presumably due to a greater concentration of soluble carbohydrates. Wager also concludes that arctic plants respire at a higher rate than those of temperate regions.

Succulent plants. Two contributions dealing with acid metabolism and respiration of succulent plants have been published by Thoday in collaboration with Jones (Mrs. K. M. Richards) (67, 68). The species examined were two succulent species of the Compositae, *Kleinia articulata* and *K. radicans.* As usual with succulents, the plants contain much malic acid. In both species there was observed the familiar diurnal fluctuation in malic acid content, this falling during the day and rising during the hours of darkness. During prolonged starvation of the stem of *K. articulata* in continuous darkness there is loss of both malate and soluble calcium, suggesting that the malic acid associated with calcium is removed and that the calcium is deposited in soluble form. The respiration rate of the stem falls rapidly during the first few days of starvation and is then maintained at a fairly constant level for a variable number of days until a rapid rise occurs correspond-

ing to injection of the intercellular spaces with cell sap. Fluctuations in respiration rate during the first few days of starvation, such as were observed by Bennet-Clark in the Crassulaceae, were not recognisable with *K. articulata,* but this might have been due to lack of uniformity in the material, since with the relatively more uniform material of *K. radicans* the starved leaves in summer showed diurnal fluctuations of carbon dioxide output very similar to those observed by Bennet-Clark in the leaves of *Crassula lactea.* This first phase of starvation was followed by a second one lasting about four days in which respiration rose and then fell. This again was followed by a third phase characterised by a very rapid rise in respiration to a maximum higher than that reached in the earlier phases, and then a less rapid fall. The last phase coincided with that injection of the intercellular spaces with sap which had previously been observed also in *K. articulata.* The three phases are not always distinct, for they may exhibit some overlapping. The third phase is reminiscent of the observation of Bennet-Clark and Bexon, mentioned later, of the increased rate of respiration which occurs when thin slices of beet root are transferred from water to juice expressed from the beet root, and Thoday and Richards regard it as highly probable that the last phase in the respiration of starved *Kleinia,* corresponding with injection of the intercellular spaces, is an example of the same phenomenon.

EFFECT OF MECHANICAL STIMULATION ON RESPIRATION

A previously unsuspected factor affecting the respiration rate of leaves has been described and investigated by Audus. This factor is mechanical stimulation. While examining the course of respiration of leaves of cherry laurel (*Prunus laurocerasus*) over a prolonged period, Audus (2) found that if leaves were removed from the experimental chamber for a few minutes during the course of an experiment, their respiration on replacement in the chamber was considerably greater than immediately before their removal. Further experiments showed that this rise was unrelated to any change in temperature or humidity to which the leaves were exposed, nor was it due to any short exposure to light. The rise in respiration rate must be attributed to the handling the leaf had suffered. The same effect can be produced by stroking or bending the leaf by a mechanical arrangement in the plant chamber, so that the leaves

are not subjected to any change of conditions other than the bending. Audus later (3) extended his observations to leaves of 15 other species, including *Laurus nobilis, Rhododendron Fargesii, Yucca gloriosa, Hedera helix, Sparganium ramosum, Typha latifolia, Polygonum cuspidatum* and *Pelargonium zonale*. The effect was noted with all of them, the observed percentage increase in the respiration rate varying from about 20% to 183%. It is important to note that Audus found (4) that in an atmosphere of nitrogen mechanical stimulation has no effect on the rate of carbon dioxide output. He concludes from this that the stimulation does not bring about an increase in the concentration of respiratory substrate, but must affect the oxidation process itself.

Later Audus (5) examined the effect on respiration of two successive stimulations and of a series of stimulations at short intervals of time. He found that the second stimulation of a pair always produced a very definite response, but although the respiration rate reached as a result of the second stimulation may be actually higher than the peak reached after the first stimulation, the net increase in the respiration rate after the second stimulation is always less than that resulting from the first stimulation if the time between the stimulations is less than 90 hours.

Audus could explain his results by supposing that the sensitivity of the leaf to stimulation is lowered by a previous stimulation. Normal sensitivity is subsequently recovered, but much more slowly than the normal respiration rate. A series of stimulations at short time intervals does not appear to reduce the sensitivity of the leaf below that resulting from the first stimulation.

Increase in the respiration rate as a result of handling has also been observed in cherry laurel leaves (26) and in potato tubers (9).

MECHANISM OF RESPIRATION

For many years the Pfeffer-Kostytchev view of the course of hexose breakdown in respiration has been accepted by most plant physiologists. As is well known, according to this view there is an intimate connexion between aerobic and anaerobic respiration or fermentation, the first stages in the two processes being the same and consisting in the breaking down of the hexoses to intermediate products which in presence of oxygen are oxidised to carbon dioxide and water, and in absence of oxygen are transformed to

other products, usually carbon dioxide and ethyl alcohol. The first stages common to both processes are now known as glycolysis, or triosis as Turner would prefer this degradation called.

In 1930 Lundsgaard proposed abandonment of the Pfeffer-Kostytchev theory of the intimate connexion between aerobic and anaerobic respiration, as a result of his experiments on the different effects of monoiodoacetate on these two processes. Turner (73) has since made an investigation on the effect of sodium monoiodoacetate on aerobic and anaerobic respiration of carrot root tissue and finds that the iodoacetate inhibits both processes in the same way, although the rate of inhibition of aerobic respiration is less than that of anaerobic respiration. This Turner ascribes to the influence of oxygen in reducing the effectiveness of iodoacetate as an inhibitor of glycolysis in living tissue. It may therefore be concluded that the different effects of monoiodoacetate on aerobic and anaerobic respiration are not discordant with the generally accepted theory of the connexion between aerobic and anaerobic respiration.

The mechanism of glycolysis has been made a subject of special study by James and a number of collaborators. With Norval (37) he had shown that pyruvic acid might be a normal intermediate, that is, a product of glycolysis, in barley, by adding killed young barley tissue to pyruvic acid (M/10 or M/15), with the result that the latter was broken down with formation of carbon dioxide and acetaldehyde on account of the action of carboxylase. Further evidence of the formation of pyruvic acid in barley respiration was later obtained (35) by poisoning roots of barley plants with 0.01% to 0.1% solutions of acetaldehyde or 0.3% solutions of certain aromatic sulphonic acids (35). Unlike untreated roots those so treated gave an ammonia-nitroprusside reaction for pyruvic acid. Moreover, from cut leaves in the dark treated with 0.2% 1-naphthol-2-sulphuric acid, pure pyruvic acid was isolated as the 2,4-dinitrophenylhydrazone. The poisons used inactivate carboxylase and so would induce the accumulation of pyruvic acid if this were formed as an intermediate product in respiration. Further evidence for the presence of carboxylase in barley was obtained by showing that it could be separated into two fractions, an insoluble apoenzyme (protein) and a soluble co-enzyme (19).

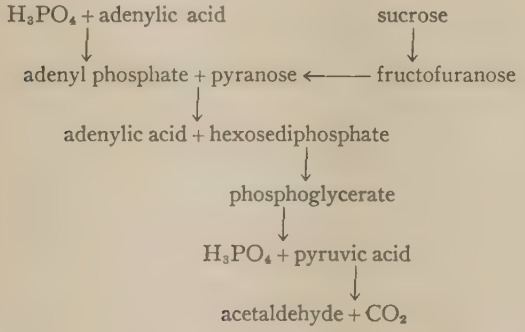
James and Arney (29) have examined the relation of the vari-

ous phosphorus compounds in barley seedlings to their respiration. The phosphorus compounds were classed as inorganic, esterified and residual. The latter, consisting mainly of phospholipoids and phosphoproteins, were determined from the difference between total phosphate and inorganic + esterified phosphate. Barley grains were germinated in respiration vessels supplied with a culture solution the phosphate content of which varied in the different vessels. Respiration was determined by the Pettenkofer method. Over the range of phosphate concentrations (0 to 0.5 g. $CaH_4(PO_4)_2H_2O$ per litre) used, no effect of this concentration on respiration was observed with whole barley grains, but sometimes the rate of respiration of embryos dissected out from endosperm was increased with increase in the concentration of phosphate. This behaviour is attributed to the presence of much phosphate in the endosperm. With addition of 4% sucrose to the culture medium, however, the respiration rate increased progressively with increase in the phosphate concentration, thus indicating that with adequate carbohydrate supply the phosphate supply can limit the rate of respiration.

In these experiments of James and Arney carbon dioxide output reached its maximum at about the fifth day from germination. At this stage various enzyme systems (amylases, invertases and carboxylase) are in excess, and with abundant sugar it is not surprising that phosphate may then limit the respiration rate. A strong correlation was found at this stage between the phosphoric ester content and respiration. With embryos dissected from the grains the phosphate ester content was found to diminish between the second and fourth days after germination, and the respiration rate diminished along with the fall in phosphate ester content. The work of James and Arney thus indicates a relationship between esterified phosphorus and respiration.

The nature of this relationship was made clearer by other work (36). Cell-free barley sap, which would contain hexose, when incubated at 30° for 48 hours in presence of thymol and 1-naphthol-2-sulphonic acid produced little or no pyruvic acid, even with added sucrose. Addition of adenylic acid to sap low in sugar content brought about the production of what appeared to be traces of pyruvic acid, but when both sucrose and adenylic acid, or both glucose and adenylic acid, were added to the sap pyruvic acid was

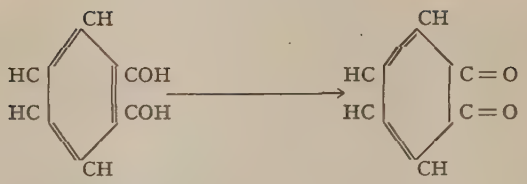
formed in readily identifiable amount, while addition of hexosediphosphate or phosphoglycerate also brought about formation of pyruvic acid. James and Bunting (30) further found that addition of adenylic acid to barley sap containing sugar and free phosphate induced a considerable increase in the production of carbon dioxide; they also found that hexosediphosphate and phosphoglycerate as well brought about increase in carbon dioxide production. From all this work the scheme shown below is suggested as representing the mechanism of glycolysis:



Thus James's view of glycolysis is that phosphorylation of hexose takes place by means of a phosphate carrier which he describes as a labile ester (adenyl phosphate in the above scheme), from which the phosphate is transferred to the hexose to form resistant esters (hexosediphosphate and phosphoglycerate) which are then degraded to pyruvic acid with reformation of phosphate and then, by the action of carboxylase, to acetaldehyde and carbon dioxide.

Although normal respiration is an oxidation process and although a number of oxidising enzymes have been recognised in plants, little was known until recently of the connexion between such enzymes and respiration. From a study of the action of one of these enzymes, catechol (or polyphenol) oxidase, in potato tubers, Boswell and Whiting (14) have come to the conclusion that this enzyme is concerned in respiration of the potatoes.

As is well known, catechol oxidase brings about oxidation of catechol and other substances with the catechol grouping, probably with the formation of *o*-diketoquinone:



Boswell and Whiting found that addition of a small quantity of 0.04 M catechol to slices of potato tuber brought about a considerable increase in their respiration, in both oxygen absorbed and carbon dioxide evolved, an increase which was followed by a fall to a level considerably below the initial rate. This fall is attributed to the oxidation product of catechol inhibiting the action of the oxidase, a view put forward in 1934 by Richter from experiments *in vitro,* and confirmed by the finding of Boswell and Whiting that addition of a further quantity of catechol has no further effect on the respiration rate. It is to be noted that the residual respiration rate depends on the thickness of the tissue slices, being lower the thinner the slice, a result which can be explained on the ground that the thicker the slice the smaller the proportion of cells in the slice which are reached by the catechol. In slices only two cells thick it may be assumed that catechol will diffuse into every cell. With such slices the residual respiration rate after inhibition of the catechol oxidase is 33% of the original rate. From these results it is concluded that two-thirds of the normal respiratory activity of the potato tuber is dependent on the catechol oxidase, while the remaining one-third is dependent on a system not involving catechol oxidase. It may be noted that potato tubers do not contain catechol, but Boswell and Whiting extracted from potato tubers a gummy material containing a phenolic *o*-dihydroxy compound which when added to potato slices brought about a rise in respiration rate. This, after a short time, remained constant at a value about 20% above normal. It would thus appear that potatoes contain an enzyme capable of oxidising phenolic *o*-dihydroxy compounds in the tissue. That this is the same enzyme as that which oxidises catechol is shown by adding the *o*-dihydroxy compound to tissue slices after the catechol oxidase has been inhibited by catechol, when no increase in respiration occurs. It is to be observed that the absorption of oxygen which takes place on the oxidation of catechol and of the *o*-dihydroxy compound in the tissue is ac-

companied by a production of carbon dioxide. It may therefore be concluded that the oxidation products of these compounds take part in some system which results in carbon dioxide production. This system, according to Boswell and Whiting, involves a dehydrase which catalyses a transference of hydrogen from a hydrogen donator to the oxidised phenolic compound which is thereby reduced to the original *o*-dihydroxy compound. It is supposed that in this action an intermediate compound is formed which splits off carbon dioxide with great ease. Thus on the view of Boswell and Whiting about two-thirds of the respiratory activity of potato tubers is brought about through the action of a system involving catechol oxidase, a phenolic compound with the *o*-dihydroxy grouping, and a dehydrase. The oxygen absorption is involved in the oxidase action, the carbon dioxide evolution in the dehydrase action.

The remaining third of the respiration is related to some system not involving catechol oxidase. Since the respiratory quotient of the whole respiration is unity, and that of the respiration connected with catechol oxidase is also unity, it follows that the respiratory quotient of the other components of respiration is also unity.

The experiments of Boswell and Whiting have been repeated by Baker and Nelson (8) who confirm the results obtained by the addition of catechol to potato slices but deny that the fall in oxygen uptake to a value below that characteristic of the tissue alone is due to the inactivation of the oxidase by catechol, since an even more pronounced fall in oxygen uptake occurs with 4-tertiary butyl alcohol which they say hardly inactivates the enzyme at all. It should be noted, however, that this last finding refers to the polyphenol oxidase from the mushroom. They therefore consider that the claim of Boswell and Whiting that two-thirds of the respiration of potato tuber depends on the oxidase loses its main support. Baker and Nelson describe experiments in which they used protocatechuic acid (4-carboxycatechol) in place of catechol. With this there results a rise in both oxygen uptake and carbon dioxide evolution so that the respiratory quotient remains close to unity, indicating a genuine rise in respiration rate, while very little quinone is produced. These facts suggest that the protocatechuic acid acted as a hydrogen carrier, being oxidised to a quinone by catechol oxidase, the quinone being then reduced by a hydrogen

donator. Inhibitors of catechol oxidase, such as potassium cyanide and 4-nitrocatechol, reduced the rate of oxygen uptake of potato slices by about 85%. That the reduction is so great is considered by Baker and Nelson to suggest that probably all the respiration of potato tissue is dependent on catechol oxidase. Boswell (13), however, in a later paper states that the proportion of the respiration due to catechol may vary according to the condition of the tissue, and would explain the difference in the value of two-thirds found by Boswell and Whiting and of 85% found by Baker and Nelson, as due to differences in the tissue used by the two pairs of investigators.

In his later work Boswell examined the oxidation of several other substances in presence of potato tuber tissue; these included a number of phenolic compounds, dihydroxymaleic acid, ascorbic acid and other organic acids, including amino-acids. Among the phenolic compounds he found that homocatechol and *p*-cresol, like catechol, inactivate polyphenol oxidase, while caffeic acid is similar in its behaviour to the polyphenol occurring in, and extracted by Boswell and Whiting from, potato tubers, there occurring a permanent increase in the rates of both oxygen intake and carbon dioxide output when it is added to the tissue. The permanence of the increases, according to Boswell, suggests that the caffeic acid takes part in a cyclic system, that is, it is alternately oxidised (when oxygen is absorbed) and reduced (when carbon dioxide is evolved). If, however, the respiratory activity of the tissue has been first partially inhibited by addition of malachite green, addition of caffeic acid brings about complete inhibition of excess carbon dioxide. This would suggest that the carbon dioxide output results from an action following an oxidation involving a dehydrase, since malachite green is an inhibitor of dehydrases. It may thus be supposed that the oxygen is absorbed in the oxidation of caffeic acid to its quinone by polyphenol oxidase and that carbon dioxide is evolved in the reduction of the quinone to caffeic acid by a hydrogen donator in a reaction catalysed by a dehydrase. Dihydroxyphenylalanine and gallic acid would also appear to form similar redox systems with polyphenol oxidase.

It will be recalled that Boswell and Whiting concluded that polyphenol oxidase controls the major part of the respiration of potato tissue, a minor part of it being controlled by some other

system. In regard to this latter, Boswell could find no evidence of the existence of a dihydroxymaleic acid oxidase forming a cyclic redox system, comparable with the polyphenol oxidase system, such as has been suggested by Szent-Györgyi, while as regards ascorbic acid, the conclusion drawn from its effects on oxygen uptake and carbon dioxide evolution was that it might be the co-enzyme of a redox system not directly involving oxygen. It was indeed found that addition of ascorbic acid to potato tuber tissue brought about a temporary rise in oxygen intake and a rise in carbon dioxide output followed by a depression, but this effect is insensitive to both sodium azide and malachite green, from which it is concluded that the oxidation is non-enzymic and metal-catalysed, probable at the surface of the tissue. More interesting results were obtained when various organic acids were added to potato tuber tissue. While the amino-acids glycine, glutamic acid and aspartic acid were without effect on either oxygen intake or carbon dioxide output, both these were increased by addition of succinic, fumaric, *l*-malic, oxalacetic, pyruvic, citric and lactic acids, and Boswell suggests that there may be a progressive oxidation following the course succinic → fumaric → malic → oxalacetic by successive action of dehydrases, for when the first three of these acids are added to tissues in which dehydrase action has been inhibited by malachite green no excess oxygen intake or carbon dioxide evolution occurs.

The decomposition of oxalacetic acid, presumably to pyruvic acid and carbon dioxide, may not be enzymic, since it is unstable and readily decomposed in the presence of proteins. As a result of work on somewhat different lines which will be discussed later, Bennet-Clark and Bexon conclude that in beet root also malic acid is broken down through oxalic acid to pyruvic acid and carbon dioxide by dehydrase action on the malic acid and subsequent decarboxylation of the pyruvic acid produced.

Boswell also examined the effect on the intake of oxygen and output of carbon dioxide by potato tissue first treated with one of the organic acids listed above and then with caffeic acid. He found that pre-treatment with an amino-acid brings about an increase in both oxygen absorbed and carbon dioxide evolved as a result of adding caffeic acid, whereas pre-treatment with a non-nitrogen-containing organic acid brings about a reduction in the excess

oxygen absorption and carbon dioxide evolution resulting from addition of caffeic acid. It has been shown that polyphenol oxidase in presence of a suitable phenol can oxidise certain amino-acids, and Boswell concludes that in respiration amino-acids are related in this way to polyphenol oxidase. Boswell's conclusions may be summed up as follows. The respiratory activity of potato tuber tissue depends on more than one oxidase system. The greater part depends on polyphenol oxidase, and this is limited by the amount of polyphenol present in the cells. Hence addition of a polyphenol such as caffeic acid increases the rate of respiration, and when polyphenol is present in excess the supply of the hydrogen donator which reduces the oxidised polyphenol (quinone) back to polyphenol becomes the limiting factor. The amino-acids act as such hydrogen donators, being themselves thereby oxidised, and so bring about an increase in the respiratory activity when caffeic acid is added in excess. Part, at least, of the respiration of potato tuber not controlled by polyphenol oxidase is associated with dehydrase actions in which non-nitrogenous organic acids such as succinic, fumaric and malic may be concerned.

Marsh and Goddard (54) also conclude that two oxidising enzymes are concerned in the respiration of root and leaves of carrot; cytochrome oxidase or a similar enzyme is responsible for most of the respiration in root and young leaves, while one insensitive to poisoning by cyanide, azide and carbon monoxide is responsible for a small part of the respiration in root and young leaves and all the respiration of mature leaves.

Respiration of the cortex of carrot root in the form of slices 0.5 mm. thick was reduced by potassium cyanide, the rate remaining practically constant after a preliminary fall, the reduction increasing from 8.7% with 1×10^{-5} M cyanide to about 80% with 3×10^{-4} M cyanide. No further significant lowering in the amount of reduction was observed with concentrations up to 1×10^{-2} M cyanide, thus suggesting that 80% of the respiration is connected with an enzyme system poisoned by cyanide. A similar inhibition of respiration of carrot root is produced by carbon monoxide, this inhibition being reversed by light. The ratio of residual respiration to respiration inhibited is proportional to the ratio of the partial pressures of oxygen and carbon monoxide; the constant in the relation residual respiration/respiration inhibited = $K \cdot P_{O_2}/P_{CO}$

is found to be of the same order as that found by Warburg for yeast. Reversible inhibition was also obtained with 10^{-3} M sodium azide.

These reactions to cyanide, carbon monoxide and azide all suggest that 80% of the respiration of carrot root is connected with cytochrome or indophenol oxidase or a similar enzyme, and not to catechol oxidase, since the inhibition of this enzyme by carbon monoxide is not reversible by light. Young carrot leaves show a similar behaviour towards cyanide, azide and carbon monoxide, but in mature leaves no inhibition of respiration was effected by these substances, from which it is concluded that some enzyme system other than cytochrome oxidase or catechol oxidase must be concerned in the respiration of mature leaves.

Henderson and Stauffer (28) examined the effect on oxygen uptake by excised tomato roots of a number of respiratory inhibitors, namely, malonate, iodoacetate, cyanide, azide and fluoride. Iodoacetate brought about the most rapid reduction in oxygen uptake, a concentration of only 3.3×10^{-5} M inhibiting respiration in four hours and a concentration of 10^{-3} M resulted in an inhibition of 73%. Cyanide and azide were also strong inhibitors, the former bringing about 79% inhibition in a concentration of 0.005 M and almost complete inhibition in a concentration of 0.01 M. Malonate and fluoride produced little inhibition except after some hours. The inhibitory action of azide and cyanide is held to indicate a cytochrome system, and the inhibitory action of iodoacetate a dehydrogenase, as concerned in the respiration of tomato roots. Succinic dehydrogenase would, however, appear to be absent, since malonate, which in animal tissues is regarded as a specific inhibitor of this, has no effect on the oxygen intake of tomato roots.

In an earlier section of this review the finding (23) has been noted that below a certain oxygen concentration there is a reduction of the respiration of the oat coleoptile to about half. It was found that treating the coleoptile with potassium cyanide in concentrations from N/3000 to N/100 reduced the rate of respiration to the same extent. It would therefore appear that cyanide and low oxygen concentration may inhibit the same system, and since the cytochrome-indophenol-oxidase system is sensitive to cyanide it is concluded that two oxidation systems are involved in the respiration of the oat coleoptile, one the cytochrome-indophenol-oxidase system and one insensitive to cyanide which might be that associated with the yellow ferment or flavin enzyme.

Brown and Goddard (17) found that the oxygen uptake by embryos removed from intact wheat grains was inhibited by hydrocyanic acid, sodium azide and by carbon monoxide, the inhibition of the last being light reversible. With 10^{-4} M sodium azide the oxygen uptake was reduced to about 25%, while 10^{-3} M hydrocyanic acid reduced it to about 9%. Since the inhibitors used inhibit the action of cytochrome oxidase, the conclusion is drawn that a large proportion of the respiration of the wheat embryo depends on cytochrome oxidase. A similar conclusion based on similar evidence was drawn in respect to barley seedlings (52, 56). Taylor (66) found also that approximately 70% of the respiratory activity of wheat seedlings and 50% of the respiratory activity of rice seedlings was inhibited by treatment with 10^{-4} M sodium azide, and concludes therefrom that at least part of the respiration of these plants is dependent on a cytochrome oxidase.

With the intention of obtaining information on the nature of the oxidising systems concerned in the respiration of barley, James and Hora (33) investigated the effect of cyanide on this process. They found that 0.002 M hydrocyanic acid brought about a depression of both oxygen absorption and carbon dioxide output of barley leaves, the effect being reversible. The depression of oxygen absorption was greater than that of carbon dioxide output. In nitrogen the rate of carbon dioxide output is about the same as in air until the output in the latter begins to rise, and addition of 0.002 M hydrocyanic does not affect the carbon dioxide evolution in nitrogen over this period. With prolonged exposure to an atmosphere of nitrogen, the rise in the respiration which would normally occur in starved leaves is very much less in an atmosphere of nitrogen, and if 0.002 M hydrocyanic acid is present as well the carbon output is still further reduced. At this stage of leaf starvation it is supposed that proteins are the principal respiratory substrate, and it would appear that lack of oxygen and presence of cyanide prevent proteolysis, a view supported by the facts that these conditions both delay or inhibit yellowing of the leaves and evolution of ammonia.

With a concentration of 0.02 M hydrocyanic acid respiration is completely and irreversibly inhibited.

From these results James and Hora conclude that an oxidation mechanism involved in respiration in barley is sensitive to cyanide,

and that while glycolysis is not directly affected, cyanide inhibits proteolysis in nitrogen.

Following up this work, James and Cragg (31) examined the expressed juice of week-old etiolated barley shoots with a view to obtaining information on the enzymic mechanism of actively respiring barley. They conclude that polyphenol oxidase is absent from barley but that a redox system involving ascorbic acid is active in this plant. Thus when ascorbic acid is added to barley juice there is a considerable increase in oxygen intake which is completely inhibited by M/500 or M/1000 cyanide. No evidence could be obtained that the oxidation of ascorbic acid is due to peroxidase, polyphenol oxidase is absent, and the method of preparation of the extract precluded the presence of cytochrome. Hence James and Cragg conclude that barley extracts oxidise ascorbic acid by means of an ascorbic acid oxidase.

James and Cragg examined the oxygen uptake resulting when a number of substances were added to the juice along with the ascorbic acid. Of 13 organic compounds only three brought about a definite increase in oxygen absorption; these were glycollic acid, lactic acid and tartaric acid, lactic acid being particularly vigorous. It is therefore suggested that the reduction of the oxidised form of ascorbic acid to the reduced form is effected by a dehydrase with an α-hydroxy acid as hydrogen donator which is thereby reduced to an α-ketonic acid. Lactic acid, for example, would be reduced to pyruvic acid. The whole redox system can then be represented by

$$AH_2 + \tfrac{1}{2}O_2 \xrightarrow[\text{ascorbic oxidase}]{\text{by}} A + H_2O$$

$$A + R \cdot CHOH \cdot COOH \xrightarrow[\alpha\text{-hydroxy dehydrase}]{\text{by}} AH_2 + R \cdot CO \cdot COOH$$

It will be observed that in the three species, potato, carrot and barley, examined by Boswell, Marsh and Goddard, and James and Cragg, respectively, different systems are held responsible for the oxidation involved in respiration.

It has been suggested that the ascorbic acid system may be concerned in the production of phosphoglycerate from hexosediphosphate (32). On incubating barley sap for 24 hours at 30° C.

with hexosediphosphate in presence of thymol and sodium fluoride, the authors observed a loss of the hexosediphosphate and an increase in unhydrolysable phosphate which they conclude is phosphoglycerate. This effect was increased by addition of ascorbic acid. Also saps clarified by centrifuging, but not incubated, with added hexosediphosphate, absorbed little or no oxygen, but rapid absorption of oxygen took place on addition of ascorbic acid, an absorption in excess of that caused by ascorbic acid alone.

Since, when sap is incubated with hexosediphosphate in presence of sodium iodoacetate, an inhibitor of triosephosphate oxidation, the latter substance accumulates, and as hexosediphosphate is not readily oxidised whereas triosephosphate is readily oxidisable, it is thought that hexosediphosphate is first broken down to triosephosphate and that this is the substance actually dehydrogenated. The work of James, Heard and James appears to be the first suggesting a possible connexion between a well known oxidation system and glycolysis.

A contribution by Bennet-Clark and Bexon (11) sheds light on the mechanism of plant respiration. They record first that a considerable increase in the respiration of thin slices of red beet root takes place when these are immersed in the expressed sap of the beet root. When undiluted sap is used the increase may be as much as 140% or more, and even sap diluted with water to 0.13% will bring about a 20% increase in respiration. This rise in respiration rate is accompanied by an increase in the respiratory quotient to a value considerably above its normal value of about unity, for the rise of carbon dioxide output is not accompanied by a corresponding absorption of oxygen. The greatest rate is reached after about half an hour and with only a slight fall is maintained for many hours. Fractionation of the sap and immersion of tissue in the various fractions indicated that organic acids, particularly malic and citric acids or their salts, were the constituents of the sap responsible for the observed effect, and this was confirmed by experiments in which slices of tissue were immersed in solutions of the sodium salts of malic, citric and succinic acids. The effects of these substances are very similar in every respect to those of expressed sap.

Bennet-Clark and Bexon next record the results of an examination of the effects of plasmolysis of the cells of beet root tissue on their respiration. These effects are quite distinct from those pro-

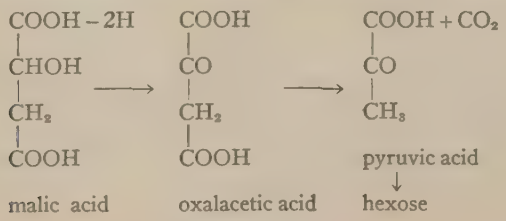
duced by applying cell sap externally to the cells. Along with plasmolysis there is at first a rapid rise in respiration followed by a fall to a steady value at a level somewhat below the original pre-plasmolysis value.

As regards the effect of sap, Bennet-Clark and Bexon point out two conditions, either of which could produce the observed results. The first of these is that the external plasmatic membrane should be more permeable than the inner plasmatic membrane, the second is that the respiratory enzyme systems should be situated on the external surface of the protoplast. Either would explain why malate, citrate or any other substance bringing about a rise in respiration rate should be more effective applied externally to the cell than in the vacuole.

Bennet-Clark and Bexon compared the loss of malate, citrate and succinate from tissue slices supplied with these, with the carbon dioxide evolved in excess of the normal amount given out by tissue respiring in water. They found that about one molecule of acid is lost for every molecule of carbon dioxide evolved. Since these acids contain four carbon atoms in each molecule, this means that only a quarter of the carbon of these acids is lost as carbon dioxide. Bennet-Clark and Bexon consider it possible that the rest of the carbon appears in hexose so that the fate of a molecule of malic acid may be expressed as

$$C_4H_6O_5 = \tfrac{1}{2}\,C_6H_{12}O_6 + CO_2.$$

An enzyme (malicdehydrogenase) in animals is known which will effect the oxidation of malic acid to oxalacetic acid, and the interaction of pyruvic acid and carbon dioxide to give oxalacetic acid or a derivative of it is known to take place in certain animal cells and bacteria, while the production of carbohydrate from pyruvic or lactic acid was demonstrated a quarter of a century ago in liver and some types of muscle by Meyerhof. The stages in the formation of hexose from malic acid might thus be represented:



Supporting evidence for the view that malic acid is not merely oxidised to carbon dioxide and water is provided by the respiratory quotient of tissue supplied with malate, for when the latter is provided in a concentration of about 0.05 N, about equal to that of malate in the sap, the respiratory quotient lies between 1.5 and 2.3 and is thus higher than a quotient provided exclusively by the oxidation of malate (1.33) and still higher than one provided by a combination of malate and carbohydrate combustion. It would, however, be given by a decarboxylation of each molecule of malic acid to provide one molecule of carbon dioxide and half a molecule of hexose. The resynthesis of carbohydrate from malate is regarded by Bennet-Clark and Bexon as comparable with that oxidative anabolism which was first demonstrated in apples by Blackman and Parija.

The effects of plasmolysis on respiration are explained as follows. In the unplasmolysed cells the malate and citrate in the vacuole diffuse across the inner plasmatic membrane to reach the respiratory enzyme system, and the slow rate of the diffusion across this membrane controls the rate of respiration. Plasmolysis of beet root cells is at first concave, and the area of the boundary between vacuole and protoplasm actually increases; later the protoplast contracts completely away from the cell wall, and the area of the internal plasmatic membrane then decreases. Assuming the amount of material in the membrane remains constant, the thickness of the membrane must first diminish and then increase.

Now the rate at which malate and citrate reaches the respiratory enzyme centres will be proportional to the concentration gradient and the area of the membrane, and inversely proportional to its thickness. In the first stage of plasmolysis, while it may be supposed there is little change in the concentration gradient, the increase in area and decrease in thickness of the plasmatic membrane will both condition an increase in the rate of diffusion of malate and citrate to the respiratory enzyme centres and so to an increase in respiration. But as concave plasmolysis gives place to contraction of the protoplast away from the cell wall, the inner plasmatic membrane decreases in area and increases in thickness and both these changes will lead to a decrease in the rate of diffusion of the malate from vacuole to enzyme systems and so to a decrease in respiration rate. On the other hand, the reduction in the volume

of the cell on plasmolysis means an increase in the concentration gradient, an increase in the rate of diffusion and increase in the rate of respiration. Bennet-Clark and Bexon, in evaluating these various factors, estimate that a reduction in the volume of the cell to one-half would bring about a reduction of 21% in the rate of diffusion of malate and citrate from the vacuole. Actually they observed initial increases in respiration on plasmolysis of about 40% or more followed by a fall to a steady value about 25% below the pre-plasmolysis level, in tissue plasmolysed with potassium chloride, calcium chloride or glucose in concentrations sufficient to reduce the volume of the protoplast to about half its initial volume.

ANAEROBIC RESPIRATION, THE PASTEUR EFFECT AND OXIDATIVE ANABOLISM

It has been mentioned earlier in this review that the Pasteur effect refers to the inhibition of sugar breakdown owing to the presence of oxygen. Since in aerobic respiration the complete breakdown of a molecule of hexose to carbon dioxide and water should result in the production of six molecules of carbon dioxide, while the breakdown of a molecule of hexose in anaerobic respiration fermentation to carbon dioxide and ethyl alcohol gives only two molecules of carbon dioxide, it may be concluded that the Pasteur effect is operative when the rate of evolution of carbon dioxide in air is less than three times the rate of evolution of this gas in nitrogen or hydrogen, that is, when the ratio of respiration (as measured by carbon dioxide output) in nitrogen to respiration in air is greater than 0.33.

During recent years a number of additions have been made to the list of tissues in which the Pasteur effect has been shown to occur. Choudhury (20) observed it in red beet root and artichoke tuber in which the ratio of respiration in nitrogen to respiration in air is about 0.75, and particularly in carrot where it was found to average about 1.36. Both Marsh and Goddard (55) and Turner (74) found a similar very considerable Pasteur effect in thin slices of carrot root. The former record that on transference from air to nitrogen the output of carbon dioxide from carrot slices increases by about 15%. Turner found the effect of transferring thin slices of carrot from air to nitrogen depended on the rate at which the tissue was respiring at the time of transference.

With disks supplied with air Turner recorded a general drift downwards in the respiration rate with time to a low level which he called the basal rate. On transference to nitrogen of tissue disks respiring at this low rate, the rate of carbon dioxide output in nitrogen was equal to, or slightly higher than, the final rate in air, but where the rate in air was high, transference to nitrogen brought about a lowering in carbon dioxide output. The rate of carbon dioxide output in nitrogen increased with time, a result agreeing with Choudhury's observations on uncut carrot roots.

No evidence for the existence of a Pasteur effect during the germination of seeds of species in which carbohydrate constituted the principal reserve could be found by Leach (45). He found the ratios of the respiratory rate in nitrogen to the respiration rate in air in *Lathyrus odoratus, Fagopyrum esculentum* and *Zea mais* were respectively, about 0.22, 0.35 and 0.25. Taylor (66), however, found quite a considerable output of carbon dioxide by wheat seedlings in nitrogen and a very big output by rice seedlings, the ratio of carbon dioxide output in nitrogen to that in air for the two species of seedlings being, respectively, 0.56 and 1.53. The values of the ratio found by Choudhury for whole potato tubers were about 0.40, a value so little above 0.33 that it cannot be regarded as evidence of the Pasteur reaction; moreover, with this tissue the interpretation of results are rendered difficult on account of the fact that no or little ethyl alcohol is recognisable as a product of anaerobic respiration.

It is well known that Blackman explained carbon dioxide evolution in nitrogen at a rate above one-third that in air not as due to a suppression of glycolysis by oxygen, but as due to oxidative anabolism by which intermediate products of the breakdown of hexose were built back into the system. Marsh and Goddard discuss their results in relation to the theory of oxidative anabolism and also to Lipman's theory, that would explain the lowered output of carbon dioxide in air as due to oxygen in respiration acting through a carrier with a fermentation enzyme, which is thereby inactivated so that the rate of glycolysis is lowered. While they hold that their results are consistent with, but do not afford proof of, the theory of oxidative anabolism, their results can be regarded as consistent with Lipman's theory only if the respiratory poisons not only inhibit respiration but also remove the inhibition of the

oxidation of the fermentation enzyme system. This would be the case if the supposed inhibition of fermentation by oxygen is catalysed by cytochrome oxidase, an enzyme which, as we have seen, they consider plays a part in the normal respiration of the carrot.

In Turner's experiments the rate of carbon dioxide output in nitrogen was more than one-third that in air, so that Turner considers there is evidence here for oxidative anabolism as hypothesized by Blackman, but the found ratios of oxidative anabolism to aerobic respiration, in terms of carbon atoms involved, are very variable. The results can, however, be explained by supposing that when the tissue is cut the rate of glycolysis is increased two or three times and the respiration rate up to five times, but that the rate of oxidative anabolism remains constant. Thus while in uncut roots the ratio of oxidative anabolism to aerobic respiration is about 3, in wounded tissue it may fall to as low a value as 0.5.

Boswell and Whiting (15) found a temporary increase in the rate of output of carbon dioxide by potato tubers kept at 29° C., but conclude that this increase results from the release of carbon dioxide not from cell respiration, but from carbon dioxide chemically bound in the tissues as bicarbonates or with amino-acids and proteins. Under anaerobic conditions, according to Boswell and Whiting, the capacity of potato tuber tissue to bind carbon dioxide is less than under aerobic conditions, for the carbon dioxide evolved on treating tissue with 10% sulphuric acid, assumed to be this bound carbon dioxide, is less under anaerobic than under aerobic conditions. Consequently, on transferring the tissue from air to nitrogen the tissue can retain less bound carbon dioxide and so some of this is released. Because the increased amount of carbon dioxide given off as a result of transferring the tubers from air to nitrogen is of the same order as the difference in the bound carbon dioxide released from slices of potato tissue treated with acid under aerobic and anaerobic conditions, Boswell and Whiting conclude that the excess carbon dioxide given off on transferring tubers to nitrogen is wholly due to a release of previously bound carbon dioxide, and that therefore there is no need to assume the existence of oxidative anabolism. It should, however, be noted that Blackman's theory of oxidative anabolism requires, not that the rate of anaerobic respiration should exceed that of aerobic

respiration, but that it should exceed only one-third of the aerobic respiration rate. The values found by Boswell and Whiting rather suggest that on transference of potato tubers from air to nitrogen there is still an excess of carbon dioxide output over one-third the previous aerobic respiration rate after allowing for the release of the bound carbon dioxide after transference to anaerobic conditions. However, the difficulty of interpreting results with potato tuber tissue has already been mentioned.

In earlier work Thomas had shown that ethyl alcohol and acetaldehyde accumulate in apples exposed to the vapour of hydrocyanic acid, the phenomenon called by him HCN-zymasis. He and Fidler (69, 70) have now made a detailed study of this. They found that healthy apples absorb hydrocyanic acid from their vapour, the rate of accumulation of the substance being determined by its external concentration up to a certain value of the latter. When the rate of cyanide accumulation exceeded a certain value aldehyde and alcohol accumulated in the tissue, the rate of their accumulation being dependent on the rate of cyanide accumulation, while the reduction of acetaldehyde to ethyl alcohol appeared to be retarded. The zymasis induced by cyanide can be explained on the view that oxidising enzymes involved in the normal respiration mechanism are poisoned by cyanide. This inhibition of enzyme activity appears to be reversible, since zymasis stops when apples containing low concentrations of cyanide are returned to air.

By measuring the alcohol + aldehyde produced, in addition to their carbon dioxide output and oxygen absorption, by apples in conditions inducing HCN-zymasis and also by apples in pure air, Thomas and Fidler have attempted to analyse the total carbon dioxide output of the former into the carbon dioxide of normal respiration and the carbon dioxide of zymasis. They find that the accumulation of alcohol and aldehyde may be accompanied by a decrease in the rate of oxygen absorption and hence of normal respiration. The rate of total carbon dioxide output may, however, be increased. It is concluded from this that hydrocyanic acid, by inhibiting the activity of the oxidation enzymes, increases the rate of breakdown of the hexose substrate. This means that the so-called Pasteur effect operates in the normal respiration of apples, and from their experimental data Thomas and Fidler conclude that the aerobic respiration of one molecule of hexose preserves about two molecules from breakdown.

CONCLUSION

From this review covering research published during the last ten years it will be seen that this period has been a very active one as far as work on respiration is concerned. Particularly has attention been directed to the mechanism of the respiratory processes, and it may be fairly claimed that real advances in our knowledge of this complex question have been made. Considerable interest has been shown in the connexion between the various oxidising systems known to exist in plant cells and repiration, so that whereas in 1935 practically nothing was known of the part played by these systems, to-day a very considerable mass of evidence has accumulated indicating the relationship between various oxidising systems and the respiratory activity of plants of different species. If the conclusions of the different workers are somewhat conflicting, this is not surprising in a line of work which has received attention so comparatively recently. In a field which is attracting so much attention we may expect that before long many of the apparent divergences will be explained.

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